THE NIH LIBRARY
NATIONAL INSTITUTES OF HEALTH

NATIONAL INSTITUTES OF HEALTH
NIH LIBRARY
FEB 2 2 1995
BLDG 10, 10 CENTER DR.
BETHESDA, MD 20892-1150

RJ
85
N277
1994

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01701-06 ESPR

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of the Impact of a Model Prenatal and Followup Program in Baltimore

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. W. Berendes	Director	OD, DESPR, NICHD
Other:	A. Herman	Visiting Scientist	EB, DESPR, NICHD
	B. Wingrove	Computer Specialist	OD, DESPR, NICHD

COOPERATING UNITS *(if any)*
Department of Health, Baltimore City (Tom Coyle)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a community based multi-risk factor intervention in a geographically defined area of Baltimore designed to improve birth outcome, especially to reduce the rate of low birth weight in the target population. The initiative for this project has come from the Baltimore City Health Department. The Baltimore City Health Department, in turn, has requested technical assistance by the staff of DESPR in the design of the intervention and the evaluation of its impact upon the target population. This is a quasi-experimental design which will compare outcome measures which are available from vital records information for birth in the target area during the period of the project with outcome measures derived from other areas of Baltimore which are similar in population characteristics. In addition there will be a process evaluation which will address smoking, drinking and drug abuse as well as compliance with prenatal care and also knowledge and attitudes about appropriate health behaviors during pregnancy.

Patients have been enrolled in this project since December 1, 1990 and approximately 500 have been enrolled thus far.

This project has become the starting point and nucleus for the Healthy Start Initiative in Baltimore which is funding by HRSA. As a result of this development, this project has been terminated and the analysis of the data are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01703-05 ESPR

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Pregnancy Outcome, Maternal Death and Child Health in Pakistan

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. W. Berendes	Director	DESPR, NICHD

COOPERATING UNITS *(if any)*
Department of Community Health, Aga Khan University (Drs. John Bryant, Fariyal Fikree, Farid Midhet, and Mehtab Karim), Special Program for Research, Reproduction and Family Planning of the WHO (Dr. Jose Villar)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has three components.

The Maternal and Infant Mortality Survey (MIMS) will provide estimates of maternal and infant mortality rates from population based samples in Karachi and the four provinces of Pakistan. In addition, the Survey will provide information on causes of maternal and infant deaths and demographic and other determinants of maternal and infant mortality.

The Pregnancy Outcome Study (POS) is a cross sectional and prospective study of pregnant women from selected katchi abadies to identify determinants of poor birth outcome in this population.

The Child Survival Study (CSS) is a follow up study of live born children from POS through two years of age to assess mortality, morbidity and physical and developmental measures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01704-04 ESPR

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stunting Among Bedouin Arab Children in the Negev, Israel

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.W. Berendes	Director	DESPR, NICHD

COOPERATING UNITS *(if any)*
Cancer Prevention Studies Branch, DCPC, NCI, NIH (M.R. Forman); Ben Gurion University of the Negev, Beer Sheva, Israel (G. Hundt)

LAB/BRANCH
Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
.05	.05	.00

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a follow up study of two sub-cohorts of Bedouin Arab children who were part of the infant feeding study of the NICHD and the Epidemiology Unit of the Ben Gurion University of the Negev study conducted between 1981 and 1983. Children from this original cohort have been identified through the school system and their height, weight and head circumference ascertained. Information has been obtained through interview, both in the school and in the home to ascertain current socioeconomic status of the family, changes over time, as well as significant morbidity. The total sample included 713 children and their mothers.

Funding for this study was obtained from the Israel Binational Science Foundation with Dr. Hundt listed as the Israeli principal investigator and Drs. Berendes and Forman as the U.S. principal investigators.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01705-01 ESPR

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Women's Lifestyles in Pregnancy Study, Analysis of Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) Name, title, laboratory, and institute affiliation)*

PI:	H. W. Berendes	Director	OD, DESPR, NICHD

COOPERATING UNITS *(if any)*

The David and Lucille Packard Foundation (Dr. Patricia H. Shiono); Center for Population, Columbia University (Dr. Virginia A. Rauh)

LAB/BRANCH

Office of the Director, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a data set is derived from a contract which terminated four years ago, entitled "Ethnic Differences" with Columbia University, New York and Northwestern University, Chicago. The project was aimed at addressing the determinants for the well known differences in rates of low birth weight among women of different ethnic groups. Through the departure of several key professional staff members involved in this project (Dr. Shiono, the original project officer, Dr. George Rhoads, a co-investigator and Dr. Leslie Cooper, who replaced Dr. Shiono as project officer) the analyses of the data have been substantially delayed.

The project included about 1100 pregnant women from six different groups (African-American, Chinese, Dominican, Mexican, Puerto Rican, White) who were below 200th percent of the Federal poverty level and were recruited at prenatal care clinics in New York and Chicago. Data were collected through two in depth interviews during the course of pregnancy which included detailed information about social, psychological, medical and lifestyle factors. Women were recruited between December 1987 and December 1989. The study is completed and analysis of data is in progress and one manuscript has been prepared and submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01802-04 CSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Coordinating Center for the NICHD Study of Early Child Care

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.K. Knoke	Computer Specialist	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

CRMC, NICHD/NIH

LAB/BRANCH
Computer Sciences Branch, DESPR

SECTION
Data Coordinating Center

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.1	PROFESSIONAL: 1.1	OTHER: 3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is a 10-site cooperative agreement to examine the effects of non-maternal care, especially during the first year of life, on the social and intellectual development of children in a ecological framework which takes into account the complex interactions of non-maternal care experiences with home and family conditions, parenting practices, and child characteristics. Over 1200 families were enrolled in the study during 1991. These families will participate in the study for 36 months.

The Data Coordinating Center (DCC) is responsible for data management and protocol monitoring. It serves as the data coordination center and is the focal point for receipt of collected data and data processing.

The DCC is currently processing 24 and 36 month data. The data are entered into computer files, edited and summarized for monitoring purposes. Routine edit reports are being distributed to the sites regularly. Project monitoring reports are distributed to the Steering Committee quarterly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01802-04 CSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Coordinating Center for the NICHD Study of Early Child Care

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.K. Knoke	Computer Specialist	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

CRMC, NICHD/NIH

LAB/BRANCH
Computer Sciences Branch, DESPR

SECTION
Data Coordinating Center

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 4.1	PROFESSIONAL: 1.1	OTHER: 3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is a 10-site cooperative agreement to examine the effects of non-maternal care, especially during the first year of life, on the social and intellectual development of children in a ecological framework which takes into account the complex interactions of non-maternal care experiences with home and family conditions, parenting practices, and child characteristics. Over 1200 families were enrolled in the study during 1991. These families will participate in the study for 36 months.

The Data Coordinating Center (DCC) is responsible for data management and protocol monitoring. It serves as the data coordination center and is the focal point for receipt of collected data and data processing.

The DCC is currently processing 24 and 36 month data. The data are entered into computer files, edited and summarized for monitoring purposes. Routine edit reports are being distributed to the sites regularly. Project monitoring reports are distributed to the Steering Committee quarterly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00325-13 EB

PERIOD COVERED
October 1, 1993 through December 31, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neural Tube Defects and Folate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Mills	Chief, Pediatric Epidemiology Section	EB, DESPR, NICHD
Other:	M.R. Conley	Computer Specialist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION
Pediatric Epidemiology Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is completed.

01

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00331-11 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes In Early Pregnancy (DIEP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Mills	Chief, Pediatric Epidemiology Section	EB, DESPR, NICHD
Other:	M.R. Conley	Computer Specialist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Cornell Univ.Med.Center, NY (L.Jovanovic); Brigham and Womens Hosp., Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ.of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ.of Washington, Seattle, WA (R.Knopp)

LAB/BRANCH

Epidemiology Branch

SECTION

Pediatric Epidemiology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Diabetes in Early Pregnancy Project was designed 1) to examine the relationship between maternal diabetic control during organogenesis and malformations in the offspring, and to identify, if possible, a specific teratogenic factor or factors in the diabetic metabolic state; and 2) to compare early fetal loss rates in women with diabetes and in non-diabetic control subjects. We found that diabetic women who came into care before the period of organogenesis achieved better results than those who came in later; but their results were still poorer than for non-diabetic control subjects. Differences in maternal glucose levels during organogenesis did not explain the malformations in the offspring of the women who were followed throughout pregnancy. These results suggest that women who enter late (and were not under medical supervision during organogenesis) probably had poor control. This resulted in malformations due to hyperglycemia or related factors. The results from the diabetic group entering early strongly suggest that other teratogenic mechanisms were present. Regarding early fetal losses, we found that diabetic women in good metabolic control were at no higher risk for spontaneous abortion than control women; the risk of loss increased dramatically as diabetic control worsened; and the overall risk of losing a pregnancy was lower than expected, only 16%. Since these primary analyses were completed, a number of related studies have been completed (see previous reports). Glycosylated protein and fructosamine assays have been performed to examine the relationship between intermediate term control and malformations. An analysis of the effect of metabolic control during pregnancy and the progression of retinopathy has been completed. A study of infection as a risk factor for early spontaneous abortion is nearing completion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 0334-11 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Low Birth Weight Across Generations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Klebanoff Research Medical Officer EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Office of the Director, DESPR, NICHD (H.W.Berendes); University of Pennsylvania (S.Katz), University of Southern California (B.Mednick)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.25	.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The original description of the correlation between birthweights of mothers and their infants was followed by the description of the association between large maternal birth weight and delivery of a macrosomic (>4000 gram) infant. Study of other fetal growth parameters, including length and head circumference, demonstrated that infants of low birth weight mothers were both shorter and lighter than infants of larger mothers, but that the infants were normally proportioned.

In ensuing studies, birth certificates of infants born in Tennessee between 1979 to 1984 were matched with those of their mothers, who were born in Tennessee between 1959 to 1966. Maternal and infant birth weights were again shown to be correlated. In addition, women who were themselves of low birth weight were up to 4 times as likely to have a small-for-gestational age infant as were women who weighed 4000-4499 grams at birth, but low birth weight women were less than twice as likely to have a preterm infant. A group of Swedish women, born from 1955 to 1965, was next studied. Women who themselves were small-for gestational age at birth were at increased risk of giving birth to both small-for-gestational age and preterm infants. Women who were preterm at birth were not at increased risk of either outcome.

Analysis of data from girls who were born in the 1960's as subjects in the Collaborative Perinatal Project and Danish Perinatal Study is currently underway in order to examine their reproductive histories. Small-for-gestational age, preterm and control girls have been located and interviewed. Hospital records of their deliveries have also been retrieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00361-08 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Child Health Supplement to the 1988 National Health Interview Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD
Other:	A. Trumble	Acting Branch Chief	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

HLB, CRMC, NICHD (P.C.Scheidt); University of North Carolina at Chapel Hill (J.Kotch); Bar Ilan University (Y.Harel); Centers for Disease Control (D.Jones); Albert Einstein College of Medicine (P.Bijur); FDA (R.Bright)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This survey provides population-based data on a nationwide representative sample of 17,200 children. Subjects include child care, family relationships, injuries, poisonings, other childhood morbidity, perinatal events, use of health services, school performance and behavior. It establishes current normative ranges for the U.S. It provides data for analysis of trends in the U.S. using the 1981 Child Health Supplement for comparisons. The survey was conducted by the U.S. Census Bureau for the National Center for Health Statistics during the 1988 calendar year with support and coordination by the NICHD. Data analysis continues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00369-06 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adverse Perinatal Events and Subsequent Injury-related Death

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.A. Klebanoff Research Medical Officer EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NIMH (M. Farmer)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several published studies have indicated that children experiencing asphyxia during the perinatal period are at increased risk of subsequent adolescent suicide. However, these studies used retrospectively ascertained data and were unable to control for the adverse social conditions that often accompany perinatal difficulties.

In the first phase of this project, the names of the approximately 55,000 children who were born during 1959-66 as subjects in the Collaborative Perinatal Project were computerized. During the second phase, the computerized names and other appropriate identifying information were submitted to the National Death Index, and all subjects who have died were identified. In the third phase, the death certificates of these subjects will be obtained and cause of death recorded. Since the Collaborative Project collected extensive data about the subjects' prenatal, perinatal, and childhood histories, it will be possible to study prospectively the relationship between adverse perinatal events and subsequent risk of death, as well as cause of death.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00373-06 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calcium Supplementation in Pregnancy for the Prevention of Preeclampsia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.J. Levine	Expert (Epidemiology)	EB, DESPR, NICHD
Others:	R. DerSimonian	Mathematical Statistician	BMSB, DESPR, NICHD
	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
University of Alabama (J.C.Hauth); Case Western Reserve U. (P.M.Catalano); Univ. of New Mexico (L.B.Curet); Oregon Health Sci.Univ. (C.Morris); Univ. of Tennessee (B.M.Sibai); The Emmes Corp. (C.L.Carter); Biomedical Research Inc. (J.Leaf)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The results of nine clinical trials suggest that supplemental calcium may prevent preeclampsia. Methodologic problems, however, limit the credibility of the results and their generalizability to other patient populations. Moreover, none of the trials has reported the outcome of systematic surveillance for urolithiasis, an important possible complication of treatment. In response to the need for a definitive evaluation of the effects of calcium supplementation, NICHD is conducting a trial at five U.S. university medical centers. Healthy nulliparous patients are randomly assigned to receive either 2 g ingested supplemental calcium daily (n=2250) or placebo (n=2250) in a double-blind study. Medication is administered beginning between 13 and 21 completed weeks of gestation and continued until the termination of pregnancy. Monitoring for the major study endpoints - pregnancy-associated hypertension and proteinuria, preeclampsia, eclampsia, and HELLP syndrome - and for urolithiasis is systematic, standardized, and thorough. It includes measurement of blood pressure, proteinuria, and hematuria at uniformly scheduled prenatal clinic visits and surveillance for hypertension and proteinuria during labor, delivery, and the first 24 hours postpartum. CPEP will have adequate statistical power to detect a reduction of 43 percent in preeclampsia risk in the calcium group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00378-05 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Mortality of Infants Weighing Less than 750 Grams

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI: M.D. Overpeck Epidemiologist EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NCHS (K.Prager); NICDD (H.J.Hoffman)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00379-05 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Analysis from the Vaginal Infections and Prematurity Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD
Other:	J.S. Read	Epidemiology Staff Fellow	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

PAMA, CRMC, NICHD (R.Nugent); NIAID (M.F.Cotch); Research Triangle Institute (A.V.Rao); Columbia University (J.Regan)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.3	.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Vaginal Infections and Prematurity (VIP) Study, jointly sponsored by NICHD and NIAID, is a prospective study designed to investigate the relationship between genital tract colonization with various microorganisms and the subsequent development of preterm birth. It also incorporates a clinical trial of erythromycin to prevent preterm birth among women colonized with *Ureaplasma urealyticum*, *Chlamydia trachomatis*, and group B streptococcus. In addition, the VIP collected a wealth of additional data on a variety of factors possibly linked to pregnancy outcome. This project will analyze these factors.

Analyses undertaken to date include the predictive value of vaginal Gram stain in the identification of group B streptococcus, the descriptive epidemiology of group B streptococcal carriage, and the relationship between reported physical activity and preterm birth. Questions under analysis include the effect of group B streptococcal colonization on pregnancy outcome, the effect of treatment with erythromycin on pregnancy outcome among women colonized with group B streptococci, the association between sexual intercourse during pregnancy and preterm birth among colonized with different genital microorganisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00382-04 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cocaine and Marijuana Use During Pregnancy and Pregnancy Outcome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Klebanoff — Research Medical Officer — EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Office of the Director, DESPR (H.W.Berendes); PAMA, CRMC (R.Nugent); Research Triangle Institute (M.Cotch); Packard Foundation (P.Shiono); University of Utah (D.Rollins)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.20	.20	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The relationship between illicit drug use and pregnancy outcome has been controversial. Self-reported drug use is notoriously inaccurate, and selective screening of blood and/or urine often results in biased ascertainment of drug use, as the reason for screening may be as important a risk factor as the drug use itself. There have been a few systematic large-scale studies of drug use at specified times during pregnancy in a defined cohort of women, and even fewer that have collected detailed data on other risk factors for adverse pregnancy outcome.

In this project, serum obtained from approximately 7,500 women on entry to the Vaginal Infections and Prematurity Study (23-26 weeks) were screened for marijuana and cocaine metabolites. In addition, serum obtained at delivery of low birth weight, preterm and a random sample of term infants was screened, as was serum obtained at 31-36 weeks from a random sample of uncomplicated pregnancies and serum obtained from women experiencing abruptio placenta and placenta previa. The Vaginal Infections and Pregnancy Study collected extensive data on a variety of confounding variables, and enabled the most thorough analysis yet of the effect of cocaine and marijuana use on pregnancy outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00383-04 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analyses of Data from the Collaborative Perinatal Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: M.A. Klebanoff Research Medical Officer EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
University of California at San Francisco (T.Newman); NEI (E.Chew);
University of Maryland (D.Nagey); Packard Foundation (P.Shiono)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.20	.20	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Collaborative Perinatal Project (CPP) represents a rich data set for the investigation of a variety of topics related to maternal and child health.

Currently active areas of investigation include the descriptive epidemiology of congenital cataract, and the association between elevated maternal serum levels of alpha fetoprotein at 14-20 weeks and subsequent stillbirth.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00384-03 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Field Trial of Oral Cholera Vaccines in Bangladesh

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Johns Hopkins School of Public Health (D.Sack); International Center for Diarrheal Disease Research, Bangladesh

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project entails continuing analyses of data derived from a large-scale, placebo-controlled field trial of B subunit-killed whole cell (BS-WC) and killed whole cell-only (WC) oral vaccines against cholera, conducted between 1985-90 in the Matlab field studies area of the International Center for Diarrheal Disease Research, Bangladesh. The trial enrolled ca. 89,000 subjects for study. Analyses of the first three years of follow-up revealed that each vaccine conferred ca. 50% protection against cholera episodes detected among patients seeking care at medical facilities. During the past year, a paper on the fourth and fifth years of surveillance, during which no further protection was evident, was prepared. In addition, an analysis of serum immune responses to V. cholerae 01 among vaccine failures revealed that such vaccinees manifested poor responses, suggesting that impaired immunity may play a role in instances of vaccine failure.

During the past year, a large-scale case-control study was conducted to determine whether infection by Helicobacter pylori, a known cause of persistent hypochlorhydria, is a risk factor for cholera in Bangladesh. Preliminary analyses indicate that the risk of clinically severe cholera is elevated in H. pylori-infected individuals, but only when such individuals lack antecedent vibriocidal immunity to cholera. Analyses are now being done to determine whether infection by H. pylori was also a determinant of vaccine failure in the field trial.

28

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00385-03 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiology of Rotavirus Infections in Bangladesh

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
James Gamble Institute for Medical Research, Cincinnati, OH (R.Ward)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project tests the hypothesis that humoral immunity to rotavirus infection, assessed in serum, is associated with the risk of rotavirus diarrhea in children aged under 2 years who reside in rural Bangladesh. Cases of rotavirus diarrhea were assembled from population-based, comprehensive surveillance of treated episodes of pediatric diarrhea in Matlab, Bangladesh during 1985-86. All rotavirus diarrhea episodes were serotyped with monoclonal antibodies after cultivating rotavirus isolates. Controls were similarly aged childred randomly selected during four surveys of the Matlab community undertaken during 1985-86. Serological correlates of natural immunity to rotavirus diarrhea were assessed by contrasting acute-phase sera collected from the cases and sera from controls. Case-control comparisons revealed that: 1) serum IgG antirotavirus antibodies were correlated inversely with the occurrence of rotavirus diarrhea, suggesting a protective relationship; but 2) protective associations were not serotype-specific. As a second goal, we sought to determine A whether breast feeding is associated with a reduced risk of rotavirus diarrhea in children under the age of 2 years. Comparisons of cases and community controls for antecedent histories of breast feeding revealed that: 1) breast feeding was associated overall with a reduced risk of rotavirus diarrhea, due to the protection conferred by exclusive but not partial breast feeding; but 2) after infancy, the direction of the relationship became reversed, with a higher risk of rotavirus diarrhea among breast-fed than non-breast-fed children.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00386-03 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of a Water-Sanitation Intervention in Egypt

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

UNICEF, Egypt

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this field project is to evaluate the impact of a combined water-sanitation intervention on the occurrence of pediatric diarrhea and other targeted outcomes in a study population residing in the Assiut governorate of Upper Egypt.

20 villages, which together included a population of approximately 10,000 persons, were randomly assigned to receive the intervention (N=10) or no intervention (N=10). The intervention consisted of provision of India Mark II tubewells and household latrines, together with an educational package stressing personal hygiene and water behaviors. To assess the impact of the intervention, all families in these villages were followed with logitudinal surveillance during a one-year follow-up period.

Although the baseline incidence of diarrheal episodes (per 1000 person-days of follow-up) in children under three years of age was similar in intervention (29) and control (29) communities, after zero-time rates of diarrhea decreased 17% ($p<.0001$) in the intervention relative to the control communities. Protective associations were slightly higher for bloody diarrhea (25%, $p<.05$) and persistent diarrhea (26%, $p<.0001$), suggesting that the intervention might have reduced not only the risk of diarrhea but also the severity of diarrhea. In contrast, no impact on the occurrence of respiratory infections was evident, indicating that the decline of diarrhea in the intervention communities was not likely to be due to ascertainment bias. It was of particular interest that intervention communities exhibited a marked increase of per capita water consumption, but that the new pumps continued to produce water that was contaminated by fecal coliforms. There data suggest that the salutory effect of the intervention may have been mediated, in part, by increased water consumption, though the water itself was fecally contaminated. Analyses are now continuing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00387-03 EB

PERIOD COVERED

October 1, 1993 through November 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sexual Intercourse during Pregnancy and Preterm Delivery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Senior Staff Fellow	EB, DESPR, NICHD
Other:	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00388-03 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Birth Weight and Infectious Disease Mortality

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Senior Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00389-03 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Epidemiology of Pediatric Shigellosis in Bangladesh

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
International Center for Diarrheal Disease Research, Bangladesh; Johns Hopkins University (F.Ahmed)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.20	.20	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this field project is to evaluate the epidemiological patterns and determinants of pediatric shigellosis occurring among residents in a research field site in rural Bangladesh who have had a documented recent exposure to a neighborhood resident having Shigella dysentery. 1934 children under 5 years of age, from 240 neighborhoods having a resident who presented for treatment of documented Shigella dysentery within 1 week of the initiation of active neighborhood surveillance, were assembled. Each of these children was followed with intensive active surveillance for one month, with a systematic schedule to ascertain symptomatic histories related to diarrhea, and to collect fecal specimens for microbiological characterization. Analyses have demonstrated that the occurrence of Shigella diarrhea was related to age (being highest in the third year of life), nutritional status (being higher in stunted children), season (being lowest during the monsoon season in the summer months), and breast-feeding status (being lower among breast-fed children, even up to 36 months of age). The presence of a latrine was not related to the risk, due to the fact that the presence of an unsanitary hanging latrine elevated the risk, while the presence of a sanitary latrine slightly depressed the risk. The lower risk of shigellosis in breast-fed than in non-breast fed children was almost entirely attributable to the increase in risk of shigrlosis which occurred during the initial 3 months following cessation of breast feeding. When host and environmental factors are considered conjointly as predictors of pediatric shigellosis, only nutritional stunting, age, and feeding mode dominate as risk factors. Vitamin A status was not related to the risk of shigellosis. Future analyses will focus on determinants of persistent diarrhea among Shigella-infected children.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00390-03 EB

PERIOD COVERED

October 1, 1993 through February 1, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Risk Factors for Stillbirth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
Other:	S. Cnattingius	Visiting Scientist	DESPR, NICHD

COOPERATING UNITS *(if any)*

NCHS (J.Kiely)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00392-03 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fatal Injuries to U.S. Infants, 1983-1987

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.A. Brenner	Staff Fellow	EB, DESPR, NICHD
Other:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD
	H.W. Berendes	Director	DESPR, NICHD
	A.C. Trumble	Computer Specialist	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The National Center for Health Statistics linked birth and death records for U.S. infants provides the first opportunity to assess the demographic, socioeconomic and prenatal risk factors associated with deaths due to injuries in infants less than one year of age. U.S. studies of childhood deaths have been limited to the extremely sparse information available on standard death certificates. Both descriptive and risk factor analysis of infant deaths for 1983-1987 are being prepared for publication.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00393-03 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Trends in Death Rates from Drowning among Children, 1971-1988

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.A. Brenner	Staff Fellow	EB, DESPR, NICHD
Other:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Johns Hopkins University (G.Smith)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

National mortality data published by the National Center for Health Statistics and population data from the U.S. Bureau of the Census were used in calculating age-, race-, and sex-specific drowning rates for 1971 through 1988. Time trends were analyzed using Poisson regression techniques. Results showed that over this eighteen year period drowning rates dropped sharply in older children, declined only slightly in toddlers and actually increased in infants (+1.6% per year in those under age one).

Drowning rates in toddlers have changed little over time despite the availability of effective prevention strategies such as pool fencing. In older ages drowning rates have declined dramatically despite the lack of clear preventive initiatives. Prevention interventions targeted specifically at the infant and toddler age groups should receive priority.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 00801-19 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies Based on the Medical Birth Registries of Norway and Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD
Other:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

NIDCD (H.Hoffman); Univ. of Bergen, Norway (P.Bergsjo, L. Irgens); Univ. of Trondheim and Natl.Inst.of Publ.Hlth., Oslo, Norway (L.Bakketeig, A.Arntzen); Uppsala Univ. (G.Lindmark, O.Meirik).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies have focused on: 1) the relation of the quality of medical care to the risk of perinatal death, 2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, 3) perinatal mortality in relation to order of birth and size of sibship, 4) epidemiologic risk factors for preterm birth, 5) epidemiologic risk factors for small-for-gestational age births, 6) contribution of multiple births to perinatal mortality rates, 7) size at birth as measured by head circumference, crown-heel length, and birth weight in relation to gestational age and perinatal mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00861-12 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

NIDCD (H.Hoffman); U.Trondheim, Norway (G.Jacobsen, L.Bakketeig); U. Bergen, Norway (P.Bergsjo, T.Markestad); U.Uppsala, Sweden (G.Lindmark, G.Ahlstein); Vanderbilt U. (G.W.Reed); U. Alabama at Birmingham (R.Goldenberg, K.Nelson)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a prospective study of risk factors associated with intrauterine growth retardation. Recruited were pregnant women before 17 weeks gestation at the University of Alabama in Birmingham and University of Trondheim, Norway (in collaboration with the Universities of Bergen and Uppsala) for the period of January 1986 through June 1988. The children are followed for five years to monitor physical growth and behavioral development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00872-09 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Associated with Premature Births: Missouri Follow-Back Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD
Other:	H.W. Berendes	Director	OD, DESPR, NICHD

COOPERATING UNITS *(if any)*
NIDCD (H.J.Hoffman); State Center for Health Statistics, Missouri Dept. of Health (G.Land, W.Schramm, J.Stockbauer, V.Pierson, B.Boley)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective is to obtain more accurate information relating to the very low birth weight (VLBW) infant, <1500 grams, than is now available from the United States vital records. This objective will be accomplished by the following: 1) mailing or administering a questionnaire to mothers of VLBW infants, mothers of fetal deaths, and a sample of mothers of moderately LBW infants (1500-2499 grams) and normal birth weight infants ($\geq$2500 grams) in order to obtain and verify information from the prenatal, perinatal, and post-neonatal periods; 2) conducting telephone follow-up interviews on non-respondents and incomplete respondents, and a 10 percent sample of study mothers to obtain and/or verify information on the questionnaires; 3) developing procedures for abstracting information from hospital and physician records, including otherwise unavailable or missing information on morbidity, lifestyle, and socioeconomic indicators of the study subjects; and 4) preparing an edited data tape for NICHD. In addition, mortality and results of follow-up evaluations will be available through the first year of life for this birth cohort.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02500-02 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
ETEC Seroepidemiology Evaluations in Alexandria, Egypt

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD
Other:	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
NAMRU-3 Unit, Cairo, Egypt (A.Mortagy, B.Kay, J.Murphy, M.Kleinosky); University of Alexandria (A.Mourad, M.Gaafar, M.Shasly); University of Goteborg (J.Holmgren, A-M.Svennerholm)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Enterotoxigenic Escherichia coli (ETEC) diarrhea is hyperendemic among young Egyptian children, with an estimated cumulative incidence of 2.4 symptomatic ETEC episodes from birth to 36 months, and a balanced distribution of toxin phenotypes. These features make it logical to develop a field site for the evaluation of ETEC epidemiology and ETEC vaccines in Egypt. Recently, we have begun a collaborative project to develop a site in Abees (near Alexandria) for the study of pediatric diarrhea.

This project is following a pediatric cohort with the following aims: 1) to determine the age-specific incidence rate of ETEC diarrhea from birth to 35 months, by toxin and colonization factor (CFA) phenotype; 2) to ascertain the strength of attribution of diarrheal symptoms to fecal isolation of ETEC; 3) to evaluate the protective relationship between titers of serum IgG antibodies to ETEC toxins and CFAs and the risk of diarrhea due to ETEC manifesting these virulence factors. Work began in June, 1993 on protocol design, data form development, development of computer systems for data entry and management, and preparation of manuals of procedures. In November, 1993 enrollment of subjects began. To date, approximately 90 children have entered the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02501-02 EB

PERIOD COVERED

October 1, 1993 through February 1, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Maternal Child Health Project of Assiut, Egypt

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.D. Clemens	Chief	EB, DESPR, NICHD
Other:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
	M.R. Rao	Visiting Associate	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 02502-02 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

NICHD-Health Research Board of Ireland Neural Tube Defects Study

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	J.L. Mills	Chief, Pediatric Epidemiology Section	EB, DESPR, NICHD
Other:	M.R. Conley	Computer Specialist	EB, DESPR, NICHD

COOPERATING UNITS (*if any*)

Health Research Board of Ireland and Trinity College, Ireland

LAB/BRANCH

Epidemiology Branch

SECTION

Pediatric Epidemiology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.8	PROFESSIONAL: 0.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The Epidemiology Branch (DESPR) is conducting a number of studies in collaboration with the Health Research Board and Trinity College, Ireland. These investigations are designed to determine the biochemical mechanisms by which folate reduces the risk for neural tube defects. Data and blood samples have been collected on a large proportion of Irish women delivering babies in Dublin. Samples from women whose pregnancy ended in the delivery of a child with a neural tube defect and control women whose pregnancy ended in the delivery of a normal child are being studied. Various aspects of folate metabolism and other nutritional measures are being examined. Ancillary studies are also being performed to determine whether other micro nutrients are involved in the pathogenesis of neural tube defects and how the risk for having an affected child varies from pregnancy to pregnancy in the same women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 02503-02 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

1991 Follow-up National Maternal and Infant Health Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

National Center for Health Statistics (M.D. Kogan and L.A. Fingerhut)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The 1991 Longitudinal Followup to the National Maternal and Infant Health Survey (LF-NMIHS) of children born in 1988 was analyzed to assess the causes for medically-attended non-fatal injuries treated in all settings. The LF-NMIHS is a nationally representative survey based on birth and death records for the 1988 birth cohort and includes demographic and medical data on the child and family. The findings of the injury analysis were accepted for publication by the American Journal of Preventive Medicine.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 02504-02 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methodological Review: Low Birth Weight and Infectious Disease Mortality

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Senior Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been completed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02505-02 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infectious Disease Mortality during Infancy: United States, 1987

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Senior Staff Fellow	EB, DESPR, NICHD
Other:	J. Troendle	Staff Fellow	BMSB, DESPR, NICHD
	M.A. Klebanoff	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is little information on risk factors for infectious disease mortality in infancy. Data from the NCHS 1987 Linked Birth/Infant Death data set has been analyzed in order to describe infectious disease mortality among U.S. infants as well as to identify potential determinants of infectious disease mortality during three periods: early neonatal, late neonatal, and postneonatal. The potential risk factors under study are derived from information available on birth certificates. Classification of cause of death is based on information listed on infants' death certificates. Hypotheses generated using this data set will be tested in the soon to be released NCHS 1989 Linked Birth/Infant Death data set.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02506-02 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Lack of Age-Appropriate Immunization among Infants Born in District of Columbia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Brenner	Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	B. Simons-Morton	Health Research Specialist	PRB, DESPR, NICHD
	F.Y. Lin	Research Medical Officer	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Children's National Medical Center (P.Scheidt); D.C. Commission of Public Health; D.C. General Hospital; Georgetown University; Howard University; University of the District of Columbia

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of the "NIH/DC Initiative to Reduce Infant Mortality" a program of research aimed at increasing the proportion of infants and children in the District of Columbia who are age-appropriately immunized has been developed. This program of research includes both epidemiologic and intervention (efficacy) studies. The first year (phase I) includes needs assessment and feasibility studies in addition to a prospective cohort study examining determinants of immunization status at 2-3 month of age. The findings from this phase will be used in the planning and execution of a larger cohort study as well as an intervention study in phase II.

Investigators include epidemiologists, behavioral scientists, health care providers and administrators. The program of research was developed with input from the variety of perspectives represented by the investigators and the proposed studies have the potential to benefit from a unique blend of epidemiology, behavioral science, and public health and health care practice. It is our intent to develop interventions and identify determinants that are relevant to public health practice in inner cities across the US.

This report focuses on the phase I epidemiologic studies.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02507-02 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prostaglandin Excretion in Preeclampsia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.L. Mills	Chief, PES	EB, DESPR, NICHD
Other:	R.J. Levine	Expert	EB, DESPR, NICHD
	J.D. Clemens	Chief, EB	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A disturbance of prostaglandin metabolism is currently believed to be one of the major mechanisms in the pathogenesis of preeclampsia. Most previous studies of this theory, however, have included only women who had already developed clinically overt disease. In order to investigate whether prostaglandin abnormalities precede the onset of preeclampsia, we have designed a nested case-control study using stored urine specimens prospectively collected as early as thirteen weeks gestation from women participating in the NICHD Trial of Calcium to Prevent Preeclampsia (CPEP). In this study, we will compare the urinary excretion of prostaglandin and thromboxane in preeclampsic women before the diagnosis of preeclampsia to that in normal pregnant women at comparable gestational ages. Because half of the women in the CPEP trial will be taking two grams of oral calcium daily, we will also be able to examine the effect of oral calcium supplementation on the excretion of these prostaglandin metabolites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02508-02 EB

PERIOD COVERED

October 1, 1993 through March 1, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Surrogate Measurements for Birth Weight and Gestational Age

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.G. Raymond	Senior Staff Fellow	EB, DESPR, NICHD
Other:	Nebiat Tafari	Visiting Scientist	EB, DESPR, NICHD
	James Troendle	Staff Fellow	BMSB, DESPR, NICHD
	John D. Clemens	Chief	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is completed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02509-02 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparison of Methods of Provision of Warmth to Low Birth Weight Newborns

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Tafari	Visiting Scientist	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	M.R. Rao	Visiting Scientist	EB, DESPR, NICHD
	K.F. Yu	Math Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Department of Pediatrics and Child Health, Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia (N. Tafari and C. Green-Abate)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.55	0.55	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02510-02 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diet, Maternal Nutritional Status, Blood Pressure and Fetal Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Tafari	Visiting Scientist	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	H.W. Berendes	Director	DESPR, NICHD

COOPERATING UNITS *(if any)*

Ethiopian Nutrition Institute (Z.W. Gabriel, E. Woohib)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study was sponsored by the City Council of Addis Ababa as part of health and nutritional assessment of low income families prior to planning a food supplementation program for women and children. This particular program was initiated because of sudden increase in the price of essential food items following implementation of government policies that preferred state control of production and distribution of all essential commodities.

The study of the effect of home diet on fetal growth arose from the observation that in Ethiopia, where severe deficiency in food energy is prevalent, there appears to be no corresponding deficit in fetal growth. This observation is at variance with the generally held view that dietary energy deficit during pregnancy leads to fetal growth retardation. However, several lines of recent evidence suggest that fetal growth is protected through adaptive processes. Among possible adaptive mechanisms area: 1) higher physical work efficiency; 2) greater mobilization of maternal energy reserves (fat deposits); and 3) hemodynamic adjustments to maintain adequate uterine perfusion.

Data analysis to date showed that the dietary energy deficits in low income families were associated with deficiencies in nutritional status of the gravida during the third trimester and the puerperium as reflected by reduced skinfold thickness and body mass index. Third trimester diastolic blood pressure was significantly reduced in poorly nourished gravida. Although a statistically significant difference of 180 g in birth weight was detected between the offspring of the undernourished and adequately nourished, it is not yet clear whether this difference is due to diet alone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02511-01 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunogenicity of Routine Childhood Vaccines in HIV Positive Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.S. Read	Senior Staff Fellow	EB, DESPR, NICHD
Other:	J.D. Clemens	Branch Chief	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

FDA (B.Anthony); NERI (D.Brambilla); NIAID (M.G.Fowler); Columbia University (J.Pitt); University of Illinois (K.Rich)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunogenicity of routine childhood vaccines in HIV-infected children is not well understood and may be substantially less than in non-infected children. It is important to determine the immunogenicity of such vaccines in this population and thus identify the most immunogenic vaccines and vaccine schedules in this population. Data from the Women and Infants Transmission Study (WITS) will be analyzed.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02512-01 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Birth Certificate Linkage to Growth and Health Measures using NHANESIII

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

National Center for Health Statistics (R.Kuczmarski, K.Flegal, G.Gay, J.Findlay)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The third National Health and Nutrition Examination Survey (NHANESIII) has collected descriptive data, physical measures, and biomedical samples on a representative U.S. sample of children at ages two months through six years with oversampling for blacks and Mexican Americans. We propose to link the extensive data from NHANESIII to the child's birth certificate to use the prenatal and birth information. Results of the linkage will support research efforts to do the following: 1) To determine if low birth weight or premature children have subsequent poor health as measured by biomedical markers in a representative national sample of children through age six; 2) to assess the effects of ethnic/racial and socioeconomic factors on these outcomes; 3) to develop normative growth and other biomedical scales for a U.S. representative population, and black and Mexican-American children, according to a risk profile at birth; and 4) to develop comparisons between maternal and proxy reports of children's risk factors at birth and actual measurements for use in other studies.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02513-01 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

High Status and Risk Factors Analysis of Premature High-Risk Deliveries

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

March of Dimes (J.Petrini, K.Damus)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The latest available information on mortality or survival of live born infants weighing less than 750 grams is being analyzed to determine changes since first analysis in 1983. Trends toward increasing survival of these very high risk infants suggest higher prevalence of complications related to extreme prematurity.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02514-01 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Childhood Drowning Deaths - A National Analysis of Death Certificate Information

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.A. Brenner	Staff Fellow	EB, DESPR, NICHD
Other:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Johns Hopkins University (G.Smith); Consumer Product Safety Commission (A.McDonald)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Working in collaboration with the Consumer Product Safety Commission (CPSC) information will be abstracted from death certificates for all non-boat related drowning deaths in children and adolescents less than 20 years of age. The free text portion of the death certificate often includes important information that is not included in national mortality files. Of particular importance is the specific site of the occurrence (e.g. swimming pool, lake, irrigation ditch, etc.) as interventions to prevent drowning deaths are site specific. Results from this study will provide the first in depth look at the circumstances surrounding drowning deaths at a national level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 02515-01 EB

PERIOD COVERED
October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prevention of Childhood Injuries - Phase I Injury Surveillance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.A. Brenner	Staff Fellow	EB, DESPR, NICHD
Other:	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*
Children's National Medical Center (P.Scheidt); D.C. Commission of Public Health; D.C. General Hospital; Georgetown University; Howard University; University of the District of Columbia

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As one component of the "NIH/DC Initiative to Reduce Infant Mortality" a program of research addressing the threat of injuries and neglect to infants and toddlers in the District of Columbia has been developed. The study is divided into two phases. During phone one a comprehensive, city-wide injury surveillance system will be established. This surveillance will include all injuries in the target age group (birth through two years) that result in an emergency room visit, hospitalization or death. Information will also be obtained from the Department of Family Services on substantiated cases of abuse and neglect.

The information obtained in phase I will guide the design of specific targeted interventions that will be implemented and evaluated in phase II. A randomized intervention trial of local community entities (neighborhoods or wards) is proposed in which epidemiologically based environmental interventions are applied to one group, behaviorally based interventions to prevent abuse and neglect to a second group and epidemiologic surveillance alone to a third group. As an essential component of injury prevention, injury surveillance system will continue for all three throughout phase II.

The remainder of this report focuses on the phase I surveillance activities as detailed plans for the intervention phase are dependent on the results of the surveillance and thus have not yet been developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HD 02516-01 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Bathtub Rings and Seats in Infant Drowning Deaths

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.A. Brenner	Staff Fellow	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Consumer Product Safety Commission (R.Rauchschwalbe); John Hopkins University (G.Smith)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Drowning is a leading cause of injury death among children in the United States. Recent studies have shown that, unlike other age groups, rates of death from drowning are actually increasing among infants. Approximately 40% of infant drownings occur in the bathtub. In this study we examine the involvement of bath rings and bath seats in drownings among infants and toddlers.

Bath rings were first marketed in the United States in 1981. They consist of a plastic ring, about 9.5 inches in diameter, with three or four attached legs, 6 to 8 inches in length. There are two basic variations in design. In the first the device is attached to the tub surface by suction cups located on the bottom surface of the legs. The infant either sits on the tub surface or on an optional sponge pad that fits within the ring. In the second design three legs are attached to a rubber mat that the infant sits on. The bottom surface of the rubber mat is covered with small suction cups that attach the device to the tub surface. In both designs there is usually a discernible front and the infant's legs are meant to straddle a particular bath ring leg. Bath seats were introduced in 1991. Similar to the bath ring, the seat contains the infant within a plastic ring and has plastic legs for straddling but the "bath seat" also provides a molded plastic seat for the infant to sit on.

Using data from the Consumer Product Safety Commission, we report the involvement of infant bath seats or bath rings in 15 drowning deaths and 8 near drownings. This report is the first in the medical literature alerting health professionals to the potential hazards of these products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02517-01 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

First Week Changes in Birthweight and Other Neonatal Anthropometric Parameters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Tafari	Visiting Scientist	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	M.R. Rao	Visiting Scientist	EB, DESPR, NICHD
	J.F. Troendle	Staff Fellow	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Department of Pediatrics, Addis Ababa University, Addis Ababa, Ethiopia

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.30	0.30	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Classification of newborns according to birthweight and gestational age is a useful clinical and epidemiologic tool to identify newborn infants at high risk for excess morbidity and mortality. In developing countries birthweight and gestational age cannot be measured directly. Moreover, preterm and low birth weight lose up to 10% of their birthweight during the first week. Using the statistical technique of recursive partitioning we recently demonstrated that head and chest circumference measurements accurately identified preterm, low birthweight newborns on data from Ethiopia. The rationale for employing other body measurements as surrogate for birthweight is dependent on their stability during the first week. We are currently examining the results of serial measurements of head, chest, midarm circumference, body weight and length measurements in a cohort of 600 Ethiopian newborns in order to determine their pattern during the first week.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 02518-01 EB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effectiveness of Rapid Plasma Reagin Screening in Gestational Syphilis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Tafari	Visiting Scientist	EB, DESPR, NICHD
Other:	J.D. Clemens	Chief	EB, DESPR, NICHD
	M.R. Rao	Visiting Scientist	EB, DESPR, NICHD

COOPERATING UNITS *(if any)*

National Health Research Institute, Addis Ababa, Ethiopia (D. Zewdie)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.60	0.60	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Congenital syphilis (CS) remains a major source of perinatal and infant mortality in sub-Saharan Africa and in high risk groups in the U.S. A major reason for the continued occurrence of CS is the inability of primary health care (PHC) services to provide a sustainable screening and treatment program. Dependence on centralized system of screening has been identified as the critical obstacle in the performance of the screening program. Moreover, a number of reports from the U.S. have drawn attention to the fact that current recommendations of prenatal treatment of gestational syphilis (GS) for prevention of CS may be inadequate.

As part of the national effort to improve the effectiveness of maternal and child health programs, the Ministry of Health of Ethiopia introduced a program of rapid plasma reagin "teardrop" (RPR-TD) test for screening and treatment of GS in PHC units without direct access to a medical laboratory. The RPR-TD was chosen over the standard venereal disease (VDRL) test because of several operational advantages including antigen stability and, possibility to carry out the test without the use of laboratory equipment such as the water bath, microscope or mechanical rotator. The test can be done and treatment can be given at initial contact thereby increasing patient and health care provider compliance. While a tendency towards reduction in fetal and infant mortality attributable to treatment could be demonstrated, there remained a large mortality gradient between the exposed and unexposed that appeared to be related in part to treatment failure, to diagnostic inaccuracy and to the presence of HIV co-infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00876-04 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Determinants of Childhood Poison Ingestion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
Others:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD
	P. Moyer	Computer Specialist	CSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

School of Medicine and Dentistry, University of Rochester (N.G. Hildreth, R.A. Lawrence, G.S. Liptak)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.225	0.175	0.050

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Determinants of Childhood Poison Ingestion study investigates the relationship between social and behavioral factors and risk of childhood ingestions of household cleaning and home repair and maintenance products.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00877-04 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prevention and Education in CHD Reduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
Others:	R. DerSimonian	Mathematical Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

School of Medicine, Northwestern University (P. Greenland) and School of Medicine and Dentistry, University of Rochester (N.G. Hildreth, C. Cox)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.125	0.125	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Prevention and Education in Coronary Heart Disease (CHD) Reduction study investigates issues related to the future design and testing of behavioral and educational strategies to help children and adolescents reduce risk behaviors and acquire and practice positive health behaviors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2100-03 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prenatal Care Utilization and Adverse Health Behaviors During Pregnancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
	D. Haynie	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.30	0.30	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research involves a program of studies employing behavioral and social support interventions to improve early entry to prenatal care and adherence to the visit schedule and decrease adverse health behaviors during pregnancy. The studies will focus on adolescent females who are at high risk of obtaining late or no prenatal care and particularly vulnerable to negative health behaviors (e.g., smoking, alcohol abuse, poor nutritional choices, illicit drug use) that are associated with poor pregnancy and birth outcomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2101-03 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Increasing Preventive Actions for Childhood Injury

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.A. Maiman	Chief	PRB, DESPR, NICHD
Others:	A. Crump	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD
	B. Radziszewska	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.43	0.43	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major purpose of the Increasing Preventive Actions for Childhood Injury study is to develop and test an intervention to reduce parental unsafe practices for childhood injuries in the home. A multicomponent preventive intervention for pediatric health care providers and parents will be developed and evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2107-03 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Children and Adolescent Trial of Cardiovascular Health (CATCH)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

University of Texas at Houston (G.S. Parcel); University of Minnesota (C. Perry); University of California at San Diego (P. Nader); Tulane University (L. Weber)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study is a test of the effectiveness of a multi-component, school-based intervention to modify CVD risk factors among preadolescent children. Blood pressure, blood lipids, body fatness, and behavioral risk factors are being assessed. The intervention includes curriculum, school environment, and family components. After an initial 3-year pilot phase, the study is now in the fourth year of a 4-year trial.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2108-03 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Afro-American Adolescent Girls Growth and Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)*

PI: B. Simons-Morton Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Center for Health Promotion Research, School of Public Health, University of Texas Health Science Center at Houston (W. Taylor)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the study is to assess longitudinally among Afro-American girls the pattern and determinants of body weight, body composition, and aerobic fitness, and physical activity.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2109-03 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

How Much Physical Activity Do Children Obtain During Physical Education Classes?

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B. Simons-Morton Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Center for Health Promotion Research, School of Public Health, University of Texas Health Science Center at Houston (W. Taylor)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research is to determine the extent to which physical education in a regional sample of elementary and middle schools provides students with moderate to vigorous physical activity during PE classes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 2110-03 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preventing Problem Behavior Among Middle School Students

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD
Others:	L.A. Maiman	Chief	PRB, DESPR, NICHD
	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD
	B. Radziszewska	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD
	D. Haynie	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD
	A. Crump	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Behavior and Health Research, Inc.

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.97	1.97	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of the study is to develop, implement, and test the efficacy of a multi-component program of school-based interventions in preventing problem behavior in a sample of middle school students. Interventions would be directed at the students to improve their resistance to problem behavior and toward the school environment and parent behavior to provide support for student prosocial behavior. The primary research question is, "What is the effect on the frequency of problem behavior among students participating in a special intervention that includes an orientation to middle school, nontraditional curriculum, and parent intervention compared to students in the comparison group?"

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2114-02 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

MD/Family Partnership: Education in Asthma Self-Management

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.A. Maiman Chief PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

School of Public Health, University of Michigan (N.M. Clark, D.W. Roloff, M.E. Hurwitz, M.A. Schork) and School of Medicine, Columbia University (D.R Mellins, D. Evans)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.15	PROFESSIONAL: 0.15	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major purpose of the MD/Family Partnership: Education in Asthma Self-Management study is to develop and test an intervention based on theories of self-regulation of behavior in changing the diagnosis and treatment patterns and educational behavior of physicians who treat children with asthma.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2115-02 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunization Among D.C. Infants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)*

PI:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD
Others:	B. Radziszewska	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Howard University, DC General Hospital, Georgetown University Medical Center, Children's Hospital, DC Commission of Public Health, University of DC, Research Triangle Insitute

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.35	0.35	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this program of research is to assess the determinants of non-immunization and to test the efficacy of interventions to improve immunization status of DC infants. Following a successful efficacy study, a large-scale effectiveness trial will be conducted.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2116-02 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preventing Pregnancies Among Adolescent Girls in the District of Columbia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Simons-Morton	Behavioral Scientist	PRB, DESPR, NICHD
Others:	A. Crump	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD
	D. Haynie	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Howard University Medical Center (R. Jenkins), Georgetown University Medical Center (J. Cox; P. Bush), Children's Hospital (K. Woodward), DC Commission of Public Health (P. Sharps), DC Hospital (M. Christie)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.43	0.43	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this research is to test the efficacy of a program of intensive interventions to prevent adolescent pregnancies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2117-01 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Validity of the Physical Activity Interview and Caltrac Motion Sensor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B. Simons-Morton Behavioral Scientist PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Center for Health Promotion Research, School of Public Health, University of Texas Health Science Center at Houston (W. Taylor)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to assess the validity of the Physical Activity Interviews (PAI) and Caltrac assessments of moderate and vigorous physical activity of 3rd- and 5th-grade children, compared with monitored heart rates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2118-01 PRB

PERIOD COVERED

April 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Parent-Child Conflict During the Transition to Middle School

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Radziszewska	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD
Co-PI:	D. Haynie	IRTA Post Doctoral Fellow	PRB, DESPR, NICHD
Others:	L. Maiman	Chief	PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research investigates changes in parent-adolescent conflict during the transition to middle school.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2119-01 PRB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Parenting Style and Adolescent Substance Use and Depressive Symptoms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B. Radziszewska IRTA Post Doctoral Fellow PRB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Department of Preventive Medicine, School of Medicine, University of Southern California (J.L. Richardson, C.W. Dent) and School of Public Health, University of Illinois at Chicago (B.R. Flay)

LAB/BRANCH

Prevention Research Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research investigates the role of parental supervision and monitoring, parenting style, and parent-adolescent conflict in adolescent substance use and depressive symptoms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00841-13 BB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methods for Comparing and Analyzing Data from Several Complex Surveys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD
Others:	H.J. Hoffman	Chief, Epidemiology	OD, EB, NIDCD
	M.D. Overpeck	Epidemiologist	EB, DESPR, NICHD
	Y.J. Lee	Chief	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

BB, CTS, DCPC, NCI (B. Graubard); BRB, NCI (E. Korn)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study developed statistical methods for the analysis of data from complex sample surveys and tested them empirically using the National Health and Nutrition Examination Survey I and II (NHANES). The research concentrated on ways to test hypotheses about the coefficients that derive from multiple linear regression of survey data. Existing methods which use Wald test statistics were compared to newly developed approaches based upon Bonferroni t-statistics and jackknifed Wald statistics. These regression methods have been applied to the NHANES data sets to determine if they can be used to provide ways to analyze the complex relationships of growth and nutrition.

Survey statistical methods proper for the National Health Interview Survey (NHIS) were used. Data sets from the Child Health Supplement of NHIS 1988 were also examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00850-18 BB

PERIOD COVERED

October 1, 1993 through April 3, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.A. Bryla	Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Human Learning and Behavior Branch, CRMC, NICHD (P. Scheidt); Intramural Research, Neuroepidemiology Branch, NINCDS (K. Nelson); Intramural Research, Developmental Neurology Branch (D. Hirtz)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00854-10 BB

PERIOD COVERED

October 1, 1993 through April 3, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of MCH Data from the National Longitudinal Youth Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.A. Bryla	Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Pregnancy and Perinatology Branch, CRMC, NICHD (D. McNellis); Ohio State University (F. Mott)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00855-03 BB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods for the Common Odds Ratio of a Number of Contingency Tables

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD
Others:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD
	J.F. Troendle	Staff Fellow	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.35	PROFESSIONAL: 0.35	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will develop statistical methods for estimating the standardized risk difference and the common odds ratio for a number of contingency tables. The optimality properties of the developed methodology will be investigated and established. The likelihood equations are to be derived. The weighted total, the maximum likelihood and the conditional maximum likelihood methodologies are to be examined. The study has been extended to do comparison of a number of contingency tables in the form of hypothesis testing. All these methodologies have wide applications to epidemiological studies, biomedical studies, genetics and other disciplines. Examples include testing the Hardy-Weinberg Law across strata and comparing the black-white differences in birth weight specific infant mortality curves.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00856-03 BB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical Methods for a Mixture of Subpopulations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.F. Yu	Mathematical Statistician	BMSB, DESPR, NICHD
Others:	A.A. Herman	Visiting Scientist	EB, DESPR, NICHD
	H.J. Hoffman	Chief	EB, OD, NIDCD

COOPERATING UNITS *(if any)*

Norway National Institute of Public Health (L.S. Bakketeig); National Center for Health Statistics (C.J. Krulewitch); Chung Yuan Christian University (M.S. Yang)

LAB/BRANCH
Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will examine the intrinsic difficulty of estimating subpopulation characteristics when the identities of the observations are not completely known. Hard clustering and fuzzy clustering techniques are investigated. This study will develop estimation of methodologies for the subpopulation characteristics. The new methodologies will alleviate some of the intrinsic difficulties for a heterogeneous mixture of subpopulations. Applications will be made to study the issues concerning biological heterogeneity and measurement error in the birth weight, gestational age and perinatal mortality. This study will also be applied to the investigation of intrauterine growth and pregnancy outcome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00857-03 BB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methodological Research in Mathematical Statistics and Biostatistics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Y.J. Lee	Chief	BMSB, DESPR, NICHD
Others:	J. Troendle	Staff Fellow	BMSB, DESPR, NICHD
	J. Legler	IRTA Fellow	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Hankook University of Foreign Studies (T. Park), Mayo Clinic (S. Jung), ATG (H. O'Grady)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Methodological research in statistics is motivated often by interest in methodological/theoretical statistical questions. Consulting and collaborative projects also motivate the research because existing methods and theories may be inadequate to handle their data analysis or study design.

Dr. Lee has completed a paper on missing data problems in clinical trials and submitted it for publication. Drs. Lee and Park have finished a paper on comparing two groups when outcome variables may be incomplete because of premature death, and submitted the paper for publication. Drs. Lee and Jung have finished a paper on logistic regression methods for censored data and submitted it for publication. Drs. Lee and O'Grady are working on the sample size problem for the two sample discrete Wilcoxon test statistic. Dr. Lee has undertaken methodological research for a nonparametric method for missing data problems in clinical trials.

Dr. Troendle is continuing to work on a resampling based method of multiple testing. One paper has been accepted for publication, and two papers have been submitted for publication. Dr. Troendle is now working with Dr. Legler to combine multiple testing and global testing methods.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00859-03 BB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Meta-Analytic Methods

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Rebecca DerSimonian	Mathematical Statistician BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.60	.60	.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project consists of several components and includes two research topics which continue from last year as well as two more recently developed efforts:

One component which continues from last year compares a fixed and a random effects model for combining data from a series of clinical trials. The study evaluates the performance of each method under various heterogeneity assumptions and with several scales of measurement, including the risk difference and the odds ratio as measures of effect.

Another component which also continues from last year develops and compares parametric and non-parametric tests to assess the assumption of homogeneity of effects in data from a series of trials. This component considers data from Gaussian, binomial, and poisson sampling distributions.

The third component is a recent addition to this project and considers meta-analytic methods for combining the evidence from a series of HIV seroprevalence studies to estimate HIV prevalence in a target population. Sentinel studies are typically investigations of incompletely defined cohorts which are convenient to survey but are often based on non-probability samples. Self-selection and similar issues inherent in sentinel studies makes generalizing results from a single study to a target population problematic. This research is to assess the use of formal meta-analytic methods for HIV prevalence estimation in a target population by incorporating information from several HIV sentinel seroprevalence studies.

The fourth component is also a recent addition to this project and addresses issues that pertain to the use of meta-analysis in the design and monitoring of clinical trials. This research is to evaluate the role of formal incorporation of external evidence summarized from a meta-analysis of previous or concurrent results into sample size considerations and stopping rules during the conduct of a clinical trial.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD-00859-03 BB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Meta-Analytic Methods

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Rebecca DerSimonian	Mathematical Statistician BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.60	.60	.00

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project consists of several components and includes two research topics which continue from last year as well as two more recently developed efforts:

One component which continues from last year compares a fixed and a random effects model for combining data from a series of clinical trials. The study evaluates the performance of each method under various heterogeneity assumptions and with several scales of measurement, including the risk difference and the odds ratio as measures of effect.

Another component which also continues from last year develops and compares parametric and non-parametric tests to assess the assumption of homogeneity of effects in data from a series of trials. This component considers data from Gaussian, binomial, and poisson sampling distributions.

The third component is a recent addition to this project and considers meta-analytic methods for combining the evidence from a series of HIV seroprevalence studies to estimate HIV prevalence in a target population. Sentinel studies are typically investigations of incompletely defined cohorts which are convenient to survey but are often based on non-probability samples. Self-selection and similar issues inherent in sentinel studies makes generalizing results from a single study to a target population problematic. This research is to assess the use of formal meta-analytic methods for HIV prevalence estimation in a target population by incorporating information from several HIV sentinel seroprevalence studies.

The fourth component is also a recent addition to this project and addresses issues that pertain to the use of meta-analysis in the design and monitoring of clinical trials. This research is to evaluate the role of formal incorporation of external evidence summarized from a meta-analysis of previous or concurrent results into sample size considerations and stopping rules during the conduct of a clinical trial.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00863-03 BB

PERIOD COVERED

October 1, 1993 through April 3, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Data Analysis for Vaccine Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.A. Bryla	Statistician	BMSB, DESPR, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Developmental & Molecular Immunity, NICHD (J Robbins)

LAB/BRANCH

Biometry and Mathematical Statistics Branch, DESPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was transferred to the Laboratory of Developmental and Molecular Immunity, NICHD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 01600-10 CBMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Basis of T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.E. Samelson, Medical Officer (Research), CBMB, NICHD
Others: J. Donovan, IRTA, CBMB
R. Guitian, Pre-IRTA, CBMB
N. Isakov, Adjunct Scientist, CBMB
Y. Ota, Visiting Fellow, CBMB
R.L. Wange, IRTA, CBMB
W. Wei, IRTA, CBMB

COOPERATING UNITS *(if any)*

Laboratory of Immunoregulation , NIAID, NIH (D.I.Cohen); Laboratory of Medicinal Chemistry, DCT, NCI (T. Burke and P. Roller); Biomedical Research Center, Vancouver (R. Aebersold and J. Watts); U. Western Australia (W. Langdon)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	5.3	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Engagement of the multicomponent antigen receptor in T cells (TCR) results in rapid activation of a protein tyrosine kinase pathway. To fully understand the function of this pathway, the kinase and its substrates must be characterized. A major TCR-associated protein tyrosine kinase is ZAP-70, a protein that binds to the activated TCR. Work from our laboratory demonstrated that the tandem SH2 domains in this kinase are responsible for its interaction with TCR subunits following their tyrosine phosphorylation. We have now shown that these SH2 domains bind directly to the tyrosine phosphorylated tyrosine-based activation motifs (TAMs) found in the cytoplasmic domains of the CD3 and TCRζ chains. A peptide with the sequence of the third TAM of the TCRζ chain was synthesized with the two tyrosines replaced with difluorophosphonomethyl phenylalanine (F_2Pmp), a phosphatase-resistant analog of phosphotyrosine. This reagent, (F_2Pmp)-TAMζ_3, when introduced into T cells blocks ZAP-70 assembly with the TCR, tyrosine phosphorylation and activation of ZAP-70, and substrate phosphorylation. The reagent thus serves as a prototype for drugs that may ultimately be used therapeutically to block T cell activation. Additional studies focus on two substrates of tyrosine kinases in T cells. They are VCP, an hexameric ATPase and p120^{cbl}, a proto-oncogene, which may be involved in the ras pathway in T cells. Abnormal tyrosine phosphorylation of the cell cycle kinase, p34cdc2, has been detected in T cells that are infected with human immunodeficiency virus (HIV). Abnormal regulation of this enzyme correlates with the cell cycle arrest at G2/M and programmed cell death that can be observed with HIV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01602-10 CBMB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Intracellular Iron Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD
Co-PI: T.A. Rouault, Senior Investigator, CBMB, NICHD
Others: J. Basilion, Staff Fellow, CBMB
R. Binder, Bio-Tech Fellow, CBMB
J. Butt, Visiting Fellow, CBMB
J. Chin, Senior Staff Fellow, CBMB
J. Felsch, EBON, CBMB
T. De Russo, Adjunct Scientist, CBMB
K. Iwai, Adjunct Scientist CBMB
C. Philpott, IRTA, CBMB

COOPERATING UNITS *(if any)*

Scripps Research Institute, La Jolla, CA (D. Stout)
American Red Cross, Rockville, MD (W. Burgess)
University of Wisconsin, Milwaukee, WI (H. Beinert, C. Kennedy)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.3	7.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed at understanding the molecular basis of intracellular iron metabolism. The cis and trans elements mediating the iron-dependent alterations in abundance of ferritin and the transferrin receptor have been identified and characterized in previous years in this laboratory. Iron-responsive elements (IREs) are RNA stem-loops found in the 5' end of ferritin mRNA and the 3' end of transferrin receptor mRNA. We have cloned, expressed, and characterized an essential iron-sensing protein, the iron-responsive element binding protein (IRE-BP). The IRE-BP binds IRE's when iron levels are depleted, resulting in the inhibition of translation of ferritin mRNA and prolongation of the half-life of the transferrin receptor mRNA. The IRE-BP is 30% identical in amino acid sequence to aconitase, a mitochondrial Krebs cycle enzyme. Mitochondrial aconitase has previously been purified and crystallized and all active site residues are identical in the two proteins. Three of the active site residues are cysteines that ligate an iron-sulfur cluster that has the relatively unusual feature of containing a labile fourth iron. The IRE-BP has aconitase activity, and in vitro manipulations of iron result in changes in RNA binding. In vivo, reciprocal regulation of aconitase activity and RNA binding can be seen when the IRE-BP is over-expressed in a stable cell line. Regulation of RNA binding activity involves a transition from a [4Fe-4S] that does not bind RNA and is an active aconitase to a form that loses iron and aconitase activity. Controlled degradation of the iron-sulfur cluster reveals that the apoprotein is the physiologically relevant form of the protein in iron-depleted cells. Thus, the cluster appears to be undergoing constant disassembly, and reassembly depends on the presence of sufficient iron pools.

The main objective of this project is to completely understand the mechanism by which changes in levels of intracellular iron result in changes in RNA binding of the IRE-BP. A variety of techniques will be employed to characterize the iron-sulfur cluster and the RNA binding site of the protein. Over-expression systems will be developed and we will attempt to crystallize the IRE-BP and co-crystallize the protein with the IRE. In addition, we have confirmed that the role of a cDNA cloned at the same time as the initial IRE-BP encodes a second IRE-BP which is rapidly degraded in cells that are iron replete. We are determining the physiologic role of these two proteins through creation of cell lines that stably express the IRP. In addition, "knockouts" of these genes are being created in mice. We intend to use the "knockout" mice to fully characterize the physiology of iron regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01605 CBMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The T Cell Antigen Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PROJECT NO LONGER ACTIVE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01606-06 CBMB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Biology of Early Organelles of the Secretory Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD
Others: J. Donaldson, Senior Staff Fellow, CBMB
D. Finazzi, Visiting Fellow, CBMB
C. Ooi, NRSA, CBMB
F. Hochstenbach, Visiting Fellow, CBMB
V. Hsu, Senior Staff Fellow, CBMB
P. Peters, Visiting Fellow, CBMB

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.8	PROFESSIONAL: 5.8	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The central vacuolar system of eukaryotic cells is an interlocking membrane system composed of distinct organelles (including ER, Golgi apparatus, secretory vesicles, secretory granules, plasma membrane, endosomes, and lysosomes) and selective transport pathways. Proper functioning of this system is vital for regulating surface expression and secretion of proteins. Current work in this project is aimed at understanding three distinct aspects of the regulation and mediation of traffic within the organelle system. Work over the past year has seen a shift in our focus from the secretory pathway to the peripheral endocytic pathway. This work is divided into three subprojects: (1) The role of ARF family GTP-binding proteins and the regulation of membrane traffic into and out of the cell surface. We have identified that a distinct subfamily of ARF GTP-binding proteins appears to regulate the critical modeling events that determine the movement of membrane into the cell and back to the plasma membrane. (2) The ability of specific proteins to move into various compartments of the central vacuolar system depends upon the expression of simple targeting motifs within the primary sequences of those proteins. Two years ago, this laboratory had identified a new generic class of targeting signals based upon the presence of a double leucine. These sequences, termed dileucine motifs, are involved in the targeting of numerous membrane proteins to a variety of specific fates within the peripheral membrane system. We have been carefully analyzing the sequence, context, and structural properties of several dileucine motifs in order to begin to understand the nature of their targeting abilities and targeting specificities. (3) Over the past year, we have developed a new genetic model system in the simple yeast *Saccharomyces cerevisiae* in order to begin to understand the process of endocytosis and membrane protein turnover in this organism. This system uses the recent discovery in CBMB of a plasma membrane metal transporter whose internalization and degradation is controlled by copper. This phenomenon allows us to establish biochemical, morphological, and genetic approaches to identifying the gene products required for plasma membrane protein internalization and degradation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01607-04 CBMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein Trafficking in the Secretory Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.S. Bonifacino, Visiting Scientist, CBMB, NICHD
Others: M.D. Delahunty, IRTA Fellow, CBMB, NICHD
M.S. Marks, IRTA Fellow, CBMB, NICHD
E. Deignan, HHMI-NIH Scholar, CBMB, NICHD
H. Ohno, Visiting Fellow, CBMB, NICHD

COOPERATING UNITS *(if any)*

R. Germain, LI, NIAID
R. Rodriguez-Boulan, Cornell University Medical College

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.25	5.25	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This group investigates the mechanisms that govern the intracellular localization and trafficking of newly synthesized proteins in the secretory pathway. Studies focus mainly on two problems: (a) the assembly of multi-subunit complexes in the endoplasmic reticulum (ER) and (b) the mechanisms of protein localization to the *trans*-Golgi network (TGN).

Studies of protein assembly in the ER were done using as a model the multi-subunit class II molecules of the major histocompatibility complex (MHC). Class II MHC molecules are composed of two polymorphic chains (α and β) and a protein known as the invariant chain (I). Our previous studies had shown that various incompletely assembled forms of class II MHC molecules are largely retained within the ER, where they form large aggregates bound to the ER chaperone, BiP. More recently, we have found that similar aggregates are not merely aberrant products of incomplete assembly, but act as true intermediates in the assembly process. Analyses of the formation and dissociation of such aggregates and of the interaction of class II species with ER chaperones have provided insights into the sequential steps of the assembly process

Our previous work on the assembly of class II MHC molecules had identified a potential glycine strip motif within the transmembrane domains of the α and β chains that was required for efficient assembly in the ER. We have recently found a similar motif within the HIV-1 fusion peptide. Mutagenesis analyses of this motif show that it plays a critical role in syncytia formation and infectivity.

We have also continued our studies on protein localization and sorting within the TGN. The cytoplasmic tail of the mammalian endopeptidase, furin, was found to contain information that is both necessary and sufficient for localization to the TGN. A tyrosine-based motif (YKGL) was shown to be partially involved in signaling a TGN localization. A related tyrosine based motif (YTPL) was found to target the MHC-like molecule, Mb, to a novel endosomal compartment. These findings suggest the existence of intracellular sorting mechanisms that recognize cytoplasmic tyrosine-based motifs with distinct specificity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 01608-04 CBMB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Regulation in Response to Enviromental Stress

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Storz Staff Fellow CBMB, NICHD
Others: M. Toledano Courtesy Associate CBMB, NICHD
S. Altuvia Visiting Associate CBMB, NICHD
I. Kullik Special Volunteer CBMB, NICHD
E. Bruggemann IRTA CBMB, NICHD

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This group studies how organisms sense environmental signals and transduce the signals into changes in gene expression and cell physiology. The group has continued their characterization of the *Escherichia coli* response to oxidative stress. Two regulators are central to the response to hydrogen peroxide: OxyR, a 34 kDa transcriptional activator which is switched on by oxidation, and OxyS, a novel 109 nucleotide RNA regulator which acts as an activator and repressor of gene expression. The group has defined functional domains within the OxyR protein and elucidated the nature of OxyR-DNA recognition. Additional OxyS-regulated genes as well mutations affecting regulation by OxyS have been characterized. Using *Arabidopsis thaliana* as a model system, the group has also identified mutants defective in their developmental response to blue light.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01609-03 CBMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Localization and Dynamics of Intracellular Organelles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: . J. Lippincott-Schwartz, Senior Staff Fellow, CBMB, NICHD
Others: Nelson Cole, Pre-IRTA, CBMB
Noah Sciaky, IRTA, CBMB
Jennifer Aparicio, IRTA, CBMB
Alex Marotta, Pre-IRTA, CBMB

COOPERATING UNITS *(if any)*

George Bloom, Univ. Texas Health Sci Center
Ted Nash, Lab of Parasitic Diseases, NIAID, NIH
Hugo Lujan, Lab of Parasitic Diseases, NIAID, NIH

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	2.0	1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Unit on Organelle Biology at CBMB studies the mechanisms underlying the spatial organization, distribution and dynamics of the membranes comprising the central vacuolar system (including ER, Golgi, lysosomes and endosomes). Because processes regulating membrane traffic and membrane-cytoskeletal interactions both play fundamental roles in establishing and maintaining the optimal organization and distribution of these membranes within cells, research in this unit bridges what historically has been two largely distinct lines of research- membrane trafficking and microtubules. Over the past year this unit has focused on the ER/Golgi membrane system of higher eukaryotes as a model for investigating the specific roles played by microtubules, microtubule motors and membrane traffic components in determining the distribution of organelles. Using a combination of biochemistry, microscopy and image analysis we have found that: 1) microtubules play an important role in directing membrane transport between the ER and Golgi complex; 2) the forward and reverse membrane transport pathways connecting the ER and Golgi complex play a role in determining Golgi localization and remodeling within cells due to constitutive cycling of Golgi resident components along these pathways; 3) the microtubule motor kinesin serves as the plus end-directed motor for Golgi-to-ER transport and cycles with membrane between the ER and Golgi complex; and 4) there are signal dependent mechanisms for regulating the extent of Golgi structure and function within primitive eukaryotes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01610-02 CBMB

PERIOD COVERED

October 1, 1993, to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intracellular Metal Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.D. Klausner, Chief, CBMB, NICHD
Others: A. Dancis, Expert, CBMB, NICHD
A. Finegold, CBMB, NICHD
D. Haile, Medical Staff Fellow, CBMB, NICHD
Y. Iwai, Visiting Fellow, CBMB, NICHD
E. Rabinovich, Guest Researcher, CBMB, NICHD
R. Stearman, Guest Researcher, CBMB, NICHD
D. Yuan, Clinical Associate/Medical Staff Fellow, CBMB, NICHD

COOPERATING UNITS *(if any)*

University of Utah, J. Kaplan

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.3	5.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

One of the fundamental nutritional paradoxes that all cells must cope with is the requirement for metals that are both essential for cellular function yet extremely toxic. The toxicity of these metals, in particular iron and copper, is based upon their redox activity and their ability to directly mediate the oxidation of macro-molecules (metal catalyzed oxidation) or to indirectly damage cells via the generation of reduced oxygen intermediates. Iron poses an addition problem for cells because, in the presence of oxygen, it is found in the insoluble ferric form. This project is aimed at understanding the molecular mechanisms responsible for the uptake, sensing, regulation, intracellular distribution, and utilization of these toxic but essential metals. This work has been approached using the simple eukaryote, *Saccharomyces cerevisiae*, which allows us to apply powerful molecular and genetic tools to the identification and characterization of essential components of metal metabolism. Through this approach, we have identified a series of genes whose function define the interlocking mechanisms by which both iron and copper are acquired from the environment by eukaryotic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00610-14 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Growth, Puberty, Their Disorders: Physiology, Pathophysiology, and Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G.B. Cutler	Head	SDE, DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

(see attached)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
9.0	7.8	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to advance understanding of the mechanisms that underlie normal and abnormal puberty, and to apply this knowledge to improve existing therapy for disorders of puberty. Since somatic growth is a major determinant of the timing of pubertal onset, a further objective is to clarify the mechanisms of normal growth and of growth failure. Principle areas of laboratory investigation include the mechanisms of normal growth and puberty. Principle areas of clinical investigation include the mechanism of premature thelarche and of the gonadotropin-independent forms of precocious puberty, the developmental changes in hypothalamic regulation of gonadotropin secretion, the behavioral changes associated with normal and abnormal pubertal development, the mechanisms of prepubertal and pubertal growth, the role of pubertal sex steroids in the acquisition of normal adult bone density, the treatment of central precocious puberty with an analog of luteinizing hormone-releasing hormone (LHRH), the treatment of familial male isosexual precocious puberty with combined antiandrogen and aromatase inhibitor, the evaluation of new approaches to the diagnosis of growth hormone deficiency and to the differential diagnosis of delayed puberty, the treatment with synthetic parathyroid hormone of children with hypoparathyroidism, the treatment with growth hormone of children with Turner syndrome and with non-growth hormone-deficient short stature, and the treatment with insulin-like growth factor-1 of children with growth hormone insensitivity and of children with non-growth hormone-deficient short stature.

The principal areas of laboratory investigation include the structure and function of the regulatory sequences of the human gonadotropin-releasing hormone (GnRH) gene, molecular analysis of the gene for the luteinizing hormone receptor in familial male precocious puberty, and hormonal regulation of epiphyseal transforming growth factor-β, fibroblast growth factor, platelet-derived growth factor, and the growth hormone receptor. To examine the effects of these growth factors in vivo we are studying rabbits bearing small needles implanted into the proximal tibial epiphyses and connected to osmotic minipumps.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00615-14 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocrine–Immune Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	G.P. Chrousos	Head	SPE, DEB, NICHD
Others:	G. Mastorakos	Visiting Fellow	SPE, DEB, NICHD
	C. Tsigos	Visiting Fellow	SPE, DEB, NICHD
	E. Webster	IRTA Fellow	SPE, DEB, NICHD
	K. Zachman	Bio Lab Technician	SPE, DEB, NICHD

COOPERATING UNITS *(if any)*

P.W. Gold, E. Sternberg, Clinical Neuroendocrinology Branch, NIMH; R. Wilder, Arthritis and Rheumatism Branch, NIAMS; Gabriel Panayi, University of London, UK; M. Reincke, University of Wurtzburg, Germany.

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Pediatric Endocrinology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.5	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinically useful antagonists exist for estrogens, androgens, and mineralocorticoids. Antagonists for the glucocorticoids or the progestins with potential clinical usefulness have been discovered recently. The objective of this project is to develop and study the molecular mechanisms of action and the human applications of the antagonists for both of these classes of steroids. We have tested a prototype glucocorticoid–progestin antagonist (RU 486) developed recently by Roussel–UCLAF. This compound has strong affinities for the human glucocorticoid and progestin receptor and is devoid of agonist effects in small experimental animals. Given to nonhuman primates or man RU 486 causes prolonged elevations of plasma ACTH, cortisol and arginine vasopressin, all changes preventable by previous administration of a glucocorticoid (dexamethasone). This suggests that antiglucocorticoids could be used for challenging the hypothalamic–pituitary–adrenal axis, when clinical testing is required in patients with disorders of this axis. Antiglucocorticoid therapy of patients with severe Cushing's syndrome due to ectopic ACTH secretion or adrenocortical tumors causes remission of the clinical manifestations of hypercortisolism. RU 486 potentiated the inflammatory/immune response to a standard stimulus in intact animals, suggesting that glucocorticoids exert anti–inflammatory/immunosuppressive effects at physiological levels. We recently demonstrated that corticotropin releasing hormone (CRH) is produced locally at the site of inflammation and has profound pro–inflammatory effects at an autocrine/paracrine level. We have called this "immune" CRH. Glucocorticoids and somatostatin suppress, and RU 486 markedly augments local secretion of immune CRH at an inflammatory site. Immune CRH was found in the ovary and endometrium where it may participate in the inflammatory phenomena of ovulation, luteolysis, and menstruation. RU 486 allowed the identification of a central nervous system defect in rats prone to arthritis. In these animals the glucocorticoid response to stress–mediators is inadequate to restrain the immune system following an inflammatory insult. The actual defect is global and located at the level of the hypothalamic CRH neuron, which responds poorly to all its known stimulants, including several cytokines, as well as serotonin, acetylcholine and norepinephrine. This pathophysiologic mechanism is novel and of relevance to human arthritis and autoimmune disease. Patients with rheumatoid arthritis have defective pituitary–adrenal axis responses to inflammatory stimuli and produce excessive amounts of immune CRH in their inflamed joints. The human CRH gene contains estrogen–responsive elements in its promoter region providing an explanation for the sexual dimorphism of the higher incidence of autoimmune/inflammatory disease in women than men. CRH antagonists may be useful in the treatment of autoimmune–inflammatory disease. We found elevated levels of arginine–vasopressin (AVP) in rats prone to arthritis and patients with rheumatoid arthritis. AVP potentiated the inflammatory response of rats to carrageenin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00616–14 DEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure, Function, and Physiology of Glycoprotein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M.R. Flack	Senior Clinical Investigator	SME, DEB, NICHD
Others:	B.C. Nisula	Head	SME, DEB, NICHD
	D. Blithe	Expert	SME, DEB, NICHD
	J. Anasti	Clinical Associate	SME, DEB, NICHD
	J. Froehlich	Visiting Fellow	SME, DEB, NICHD
	C. Finch	Biotechnician	SME, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of this project is to understand the endocrinology of the human glycoprotein hormones, follicle–stimulating hormone (FSH), choriogonadotropin (CG), luteinizing hormone (LH), thyroid–stimulating hormone (TSH), and thereby to develop diagnostic and therapeutic applications for these hormones. Research advances in the current period include: First, we determined the roles of the FSH oligosaccharides. The αAsn^{52} oligosaccharide had a disproportionate role in signal transduction, but the other oligosaccharides had cooperative roles, so that as the number of oligosaccharides decreased, binding increased, and signal transduction decreased. As a result FSH lacking all its oligosaccharides behaved as a potent FSH antagonist. Second, we identified sites on FSH mediating receptor binding distinct from signal transduction. We substituted neutral or negatively charged residues for the positively charged Arg^{35} and Lys^{86} of βFSH and found they were involved in receptor binding, but not signal transduction. Isolation of discrete foci for receptor binding and signal transduction forms the basis for the development of FSH agonists and antagonists. Third, we made mutations in the human FSH receptor (hFSH–R) gene to identify sites of the hFSH–R important for receptor binding and signal transduction. Arg or Asp substitutions at Ala^{571} in the third cytosolic loop of the seven transmembrane spanning domain increased receptor binding affinity 15 fold, while maintaining signal transduction. An Ile substitution constitutively activated adenylyl cyclase activity, analogous to the TSH receptor mutations found in patients with follicular adenomas. Fourth, we isolated an LH/CG receptor clone and demonstrated its functional expression in cos7 cells. Future directions of the project will emphasize the study of the structure–function characteristics of human FSH, LH, and CG and their homologous human receptors using side–directed mutagenesis of their respective genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00618-13 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiology and Pathophysiology of the Hypothalamic–Pituitary–Adrenal Axis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G.P. Chrousos	Head	SPE, DEB, NICHD
Others:	See attached list.		

COOPERATING UNITS *(if any)*

See attached list.

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Pediatric Endocrinology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	4.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we seek to advance the understanding of the physiology and pathophysiology of the hypothalamic–pituitary–adrenal axis. The role of stress–related hormones in normal and disease states is being examined, and clinical applications for these hormones are sought. The recent discovery of the structure of corticotropin releasing hormone (CRH) and the development of sensitive assays for measuring stress–related hormones and their receptors have led to rapid progress in this field. Major progress has been made in three areas: 1) Clinical applications of CRH: An ovine CRH (oCRH) stimulation test has been developed that is useful in the differential diagnosis of adrenal insufficiency, Cushing's syndrome, and pseudo–Cushing's states (psychiatric hypercortisolism). The human CRH (hCRH) analog is useful in studying the physiology of the pituitary–adrenal axis. The oCRH stimulation test and measurement of CSF CRH have increased our understanding of the pathophysiology of Cushing's syndrome, melancholic depression, atypical depression, seasonal depression, the premenstrual tension syndrome, anorexia nervosa, the chronic fatigue syndrome, fibromyalgia, late–onset congenital adrenal hyperplasia, diabetes mellitus and rheumatoid arthritis. 2) The regulation of the axis by opioids, vasopressin, oxytocin, glucocorticoids, eicosanoids, platelet activating factor (PAF), tumor necrosis factor – alpha, interleukin–6, cholecystokinin (CCK), neuropeptide Y, benzodiazepine agonists and antagonists and thyroid hormones has been studied in vivo and/or in vitro. Neurotransmitter and feedback regulation of hypothalamic CRH secretion has also been examined in vitro and in vivo. Third trimester pregnant women and athletes have a hyperfunctional pituitary–adrenal axis in the resting state. Hypothalamic–pituitary–adrenal axis reactivity and personality traits have been correlated in developing adolescents. The hCRH gene 5' regulatory region has been cloned and sequenced and its regulation is being studied. It has 2 active promoters and responds positively to estrogens, providing a potential explantation for the sexual dimorphism of psychiatric diseases characterized by abberations in CRH secretion. 3) Role and actions of glucocorticoids: Surgical stress is associated with continuous and pulsatile secretion of ACTH. Glucocorticoid resistance is an autosomal recessive or dominant disease associated with abnormalities of the glucocorticoid receptor type II. We have elucidated the molecular pathophysiology of this syndrome by defining mutations and/or deletion of the glucocorticoid receptor gene leading to abnormal or decreased receptors in the tissues of patients. The glucocorticoid receptor type I (mineralocorticoid) is studied in several kindreds with mineralocorticoid resistance. The interaction of the glucocorticoid receptor with the heat–shock proteins is studied. Hereditary ACTH resistance is an autosomal recessive disorder characterized by pituitary glucocorticoid deficiency. We have elucidated the molecular pathophysiology of this syndrome by defining abnormalities of the ACTH receptor gene.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00621-12-DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnosis and Treatment of Growth Disorders.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. Cassorla	Head	UGP, DEB, NICHD
Others:	H. Domené	Visiting Associate	UGP, DEB, NICHD
	Y. Yu	Visiting Associate	UGP, DEB, NICHD

COOPERATING UNITS *(if any)*

D. Le Roith, Diabetes Branch, NIDDK

S. Rose, Department of Pediatrics, University of Tennessee, Memphis

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Growth Physiology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5		0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00623-11 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adrenal Physiology, Pathophysiology, and Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G.B. Cutler	Head	SDE, DEB, NICHD
Others:	(see attached)		

COOPERATING UNITS *(if any)*

J. Doppman, Radiology, CC; E. Oldfield, Surgical Neurology Branch, NINDS; J. Booth, Dept. Internal Medicine, McMaster University Medical Center; S. Radovick, F. Wondisford, Dept. Pediatrics and Internal Medicine, Harvard Medical School; D.L Loriaux, Oregon Health Sciences University

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.9	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We seek to advance understanding of the mechanisms that cause adrenal androgen secretion by the fetal adrenal zone prenatally and by the definitive adrenal cortex during adrenarche, and to improve the diagnosis and treatment of disorders that cause excess adrenal androgen or glucocorticoid secretion, such as premature adrenarche, congenital adrenal hyperplasia, adrenal neoplasms, idiopathic hirsutism, polycystic ovary syndrome, and Cushing syndrome. We also seek to clarify the pathophysiology of primary adrenal insufficiency (Addison's disease) and secondary adrenal insufficiency and to improve the treatment of these conditions.

Children with congenital adrenal hyperplasia are being enrolled into one of two projects. The first examines the adrenocorticotropin-suppressing activity of carbenoxolone, an inhibitor of 11-beta-hydroxysteroid dehydrogenase. The second employs a newly available antiandrogen and an inhibitor of androgen-to-estrogen conversion to block the action of excess androgen levels. Additionally, the potential to cure the 21-hydroxylase deficiency form of congenital adrenal hyperplasia by adrenal transplantation or gene therapy is being explored in a newly recognized animal model of this disorder.

Patients with Cushing syndrome are being studied by several new diagnostic methods to determine the relative diagnostic efficiency of these new methods compared to the old. Oncogene expression in corticotropinomas is also under study in an attempt to elucidate the molecular basis of Cushing disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00626-06-DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994.

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Progesterone Action in Reproduction.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. K. Nieman	Senior Investigator	URM, DEB, NICHD
Others:	D. Johri	Visiting Fellow	URM, DEB, NICHD
	F. Sharara	Visiting Associate	URM, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Gynecologic Research

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been discontinued.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00627-0~~4~~5 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Glycoprotein hormones: oligosaccharide structure and function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Blithe	Head	UG, SME, DEB, NICHD
Others:	M. Nemansky	Visiting Fellow	UG, SME, DEB, NICHD
	A. Renwick	Adjunct Scientist	UG, SME, DEB, NICHD
	E. Moy	Clinical Associate	UG, SME, DEB, NICHD
	C. Lyons	Bio Lab Technician	UG, SME, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Glycobiology, Section on Medical Endocrinology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of the project is to increase our understanding of the structure-function relationships and glycobiology of the oligosaccharide moieties on glycoprotein hormones. The molecules currently under investigation are, human chorionic gonadotropin (hCG), gonadotropin free α subunits associated with pregnancy or malignancy, and pituitary free α subunits secreted throughout the normal menstrual cycle. We have shown that free α subunit purified from pregnancy urine stimulates secretion of prolactin from primary cultures of human decidual cells in a dose-dependent manner. This finding indicated that free α is a glycoprotein hormone with potential functions that are independent of heterodimeric gonadotropins. We further found that free α can play a role in recruitment of endometrial stromal cells for decidualization. This observation expands the function of the free α subunit into areas of reproductive physiology that occur during the luteal phase of the menstrual cycle and may suggest involvement of free α in implantation.

Carbohydrate modifications, resulting in a variety of branched oligosaccharide structures, occur on all glycoproteins prior to secretion. These modifications can affect virtually every aspect of the molecule's behavior, including receptor binding and signal transduction, yet the underlying regulatory mechanisms remain elusive. We have investigated functional aspects of the oligosaccharide moieties on free α, demonstrating that specific types of carbohydrate modifications on free α can either prevent or facilitate combination with hCG-β to form intact hormone. We have found that the free α subunits that are located in fetal compartments in high concentrations during early pregnancy exhibit physical properties that are similar to those of the free α subunit isolated from pregnancy urine. We have shown that the oligosaccharide moieties on this early pregnancy free α function to maintain the molecule in an uncombined form in placental compartments that also contain high amounts of combinable free β-subunit.

We have identified differences in the oligosaccharide moieties of free α and hCG as a function of gestational development; in particular, the glycosylation of free α changes dramatically as a function of gestational age. Molecules produced in late pregnancy are more highly branched and are much more extensively fucosylated than those of early pregnancy. We plan identify the time frame in pregnancy during which the glycosylation changes occur and to examine how the differences observed in glycosylation affect the bioactivity of these molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00628-05 DEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological roles and mechanisms of action of insulin-like growth factors (IGFs)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	C.A. Bondy	Senior Clinical Investigator	SGM, DEB, NICHD
Others:	O. Adesanya	Medical Staff Fellow	SGM, DEB, NICHD
	E. Chin	Senior Staff Fellow	SGM, DEB, NICHD
	C. Mitchell	Pre-IRTA Fellow	SGM, DEB, NICHD
	R. Reinhardt	Medical Staff Fellow	SGM, DEB, NICHD
	E. Wang	NRSA Fellow	SGM, DEB, NICHD
	J. Wang	Special Volunteer	SGM, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Growth and Metabolism

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	3.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Section carries out both basic and clinical research on the biological role and mechanisms of action of peptide growth factors such as insulin-like growth factors-I and -II (IGFs-I and -II) in embryonic development and reproduction. These particular growth factors have critical roles in development as proven by the fact that targeted deletion of IGF-I or IGF-II gene expression results in dwarfism and infertility - although how IGF deletion causes these defects is not yet understood. Molecular, immunological and radioligand probes are used to analyze the cellular sites of synthesis and action of these growth factors during embryonic development as well as in different physiological and pathophysiological situations in which IGFs have key roles.

This Section has made a number of important and novel discoveries in the past year. 1) The insulin receptor-related receptor (IRR) is a newly discovered member of the IGF receptor family whose ligand and biological role are still unknown. This lab has shown that during embryonic development, the IRR is exclusively and synchronously co-expressed with the high-affinity nerve growth factor (NGF) receptor, TRK, suggesting that these two receptors are physically or functionally linked in determining the survival of sensory and sympathetic neurons - and that the IRR ligand is likely to be a novel neurotrophin. 2) The section has provided the first evidence that IGFs-I and -II cross the blood brain barrier with potency at least ten-fold greater than insulin, suggesting that systemic IGF levels may play an important role in relaying information concerning peripheral metabolic-nutritional status to brain regulatory centers. 3) The group has shown that production of components of the high molecular weight IGF binding complex, which is responsible for maintaining stable IGF levels in the circulation - is segregated into distinct hepatic cellular compartments which are differentially regulated by growth hormone. 4) of great importance for women's reproductive health, we have found that IGF-I appears to stimulate endometrial growth in the follicular phase of the menstrual cycle, while IGF-II appears to trigger endometrial decidualization in the secretory phase. These findings have very significant clinical implications in terms of the potential utilization of IGFs to selectively stimulate anabolic processes in the nervous and reproductive systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00631-05 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neuroendocrine Control of the Stress Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G. Aguilera	Head	SEP, DEB, NICHD
Others:	A. Kiss	Visiting Scientist	SEP, DEB, NICHD
	X. Luo	NRSA Fellow	SEP, DEB, NICHD
	C. Rabadan-Diehl	IRTA Fellow	SEP, DEB, NICHD
	G. Makara	Visiting Scientist	SEP, DEB, NICHD

COOPERATING UNITS *(if any)*

S. Lolait, LCB, NIMH, NIH

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.7	3.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research has focused on the mechanism of regulation of hypothalamic and pituitary function during stress. The studies have shown that the level of response of the pituitary corticotroph is determined by differential regulation of the hypothalamic regulators corticotropin releasing hormone (CRH) and vasopressin (VP) and the sensitivity of the glucocorticoid feedback. While the inhibition of the HPA axis during osmotic is associated with stimulation of VP secretion by magnocellular neurons of the paraventricular (PVN) and supraoptic nuclei of the hypothalamus, physical–psychological stress paradigms associated with corticotroph hyperresponsiveness show activation of CRH and CRH/VP parvicellular neurons in the PVN.

Binding studies using [^{3}H]VP and anterior pituitary membranes from rats under osmotic stress (water deprivation and 2% saline intake) or physical–psychological stress (immobilization or i.p. hypertonic saline injection) showed a good correlation between changes in pituitary VP receptors and corticotroph responsiveness. Northern blot analysis of VP receptor mRNA in pituitaries from chronically stressed rats revealed changes in mRNA levels parallel to those in VP binding. In contrast to the findings in chronic stress, continuous exposure of the pituitary to increased levels of VP by surgical shunting of magnocellular VP to the hypophyseal portal circulation caused corticotroph desensitization and pituitary VP receptor downregulation. These data suggest that regulation of VP receptor number plays a critical role in the regulation of corticotroph responsiveness during chronic stress.

Studies on the expression of CRH receptors in the brain revealed that while CRH binding and CRH receptor mRNA are undetectable in the PVN under basal conditions, they markedly increase during stress with expression in parvicellular cells after physical psychological stress and in magnocellular cells after osmotic stimulation. These data suggest an autoregulatory role for CRH in the control of PVN function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD–00632–05 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Physiological Actions of the Renin–Angiotensin–System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Aguilera	Head	SEP, DEB, NICHD
Others:	B. Akbasak	Visiting Scientist	SEP, DEB, NICHD
	A. Kiss	Visiting Scientist	SEP, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section Endocrine Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study physiological and pathological aspects of the renin–angiotensin system, with emphasis on its role on circulatory homeostasis and development. The expression of AII receptor subtypes, type–1 (AT$_1$), including AT$_1$a and AT$_1$b, and type–2 (AT$_2$) were studied during development in the mouse. While AT$_1$a was present in the adrenal gland, kidney, liver, ovary, testes, adipose tissue, lung, heart and brain of adult mice, AT$_1$b was found only in the adrenal, and very low levels in testes and brain. In contrast to the marked changes in expression of AT$_2$ receptors during development, similar proportions of AT$_1$a and AT$_1$b in the different tissues were found in fetal and adult mice. Studies in the rat using binding autoradiography and in situ hybridization showed converse changes in the expression of AT$_1$ and AT$_2$ in the kidney during post–natal development. Abundant AT$_2$ receptor expression in the metanephric mass and advancing tubules and ampule of the ureteric bud of the immature kidney, decreased gradually through day–14, disappearing after completion of nephrogenesis. In contrast, AT$_1$ expression was very low 2 days after birth and was confined to the cortex and juxtamedullary boundary. AT$_1$ expression progressively increased in correspondence to newly developed glomeruli and extended from the juxtamedullary boundary to the whole extent of the outer medulla. The temporal pattern of expression of the receptor subtypes suggests that while AT$_2$ are involved in cell proliferation and early differentiation, AT$_1$ receptors have a dual role in nephron differentiation and later in renal function.

Studies on the brain renin–AII system focused on the role of AII in the regulation of the hypothalamic–pituitary–adrenal axis and vasopressin secretion. In situ hybridization studies showed AT$_1$ receptor mRNA in periventricular and parvicellular perikarya of the paraventricular nucleus of the hypothalamus (PVN). Autoradiographic studies showed AII binding in areas corresponding to the mRNA location, but in addition AII receptors were clearly present in afferent fibers to the magnocellular division of the PVN. At the cellular level, double labelling in situ hybridization showed that AT$_1$ receptor mRNA was located in corticotropin releasing hormone (CRH) neurons, but not in vasopressin and oxytocin neurons. In addition, central administration of AII increased CRH mRNA, but not VP mRNA levels in the PVN. The data suggest that while AII stimulates VP release indirectly through modulation of afferent innervation, AII has a direct regulatory effect on the CRH neuron.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00633-04 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ovarian Folliculogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Lawrence Nelson, M.D.	Sr. Clinical Investigator	URE, SGR, DEB, NICHD
Others:	J. Anasti	Clinical Associate	URE, SGR, DEB, NICHD
	R. Maity	Visiting Fellow	URE, SGR, DEB, NICHD
	S. Nair	NRSA Fellow	URE, SGR, DEB, NICHD
	Z. B. Tong	Visiting Fellow	URE, SGR, DEB, NICHD

COOPERATING UNITS *(if any)*

R. Caspi, Laboratory of Immunology, NEI; J. Dean, Laboratory of Cellular and Developmental Biology
Mammalian Developmental Biology Section, NIDDK

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Gynecologic Research, Unit on Reproductive Endocrinology

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project seeks to understand the physiology and pathophysiology of ovarian follicle development and function, and to thereby develop clinical diagnostic and therapeutic applications for women with ovulatory disorders. During this period we have focused on premature ovarian failure, a condition which prematurely terminates fertility in 1% of women. We have particular interest in autoimmune-mediated ovarian failure, and seek to improve understanding of this condition through clinical research and through basic research employing a murine model of autoimmune ovarian failure. At one time premature ovarian failure was assumed to be caused by depletion of ovarian follicles, and, like menopause, to be an irreversible condition. We continue to collect evidence to refute this view. A major goal of ours has been to define the mechanism causing follicle dysfunction in these women. By pelvic ultrasound we imaged an antral follicle in over 40% of our patients with premature ovarian failure. However, the follicles were dysfunctional as evidenced by the lack of strong correlation between follicle diameter and serum estradiol and progesterone that is found in normal women. We biopsied antral follicles in 6 women to determine if it was autoimmune oophoritis or some other mechanism impairing follicle function. Strikingly, we found luteinized graafian follicles in all cases. Inappropriate luteinization of graafian follicles appears to be the major pathophysiologic mechanism preventing normal follicle function in these women. There are well recognized increases in reproductive pathology that develop in maturing women. Our findings raise the distinct possibility that these result from a decline in follicle number that reduces negative feedback on luteinizing hormone rather than an intrinsic defect within the remaining primordial follicles. If true, this would have enormous public health implications. We did not find autoimmune oophoritis in any of 6 ovarian follicle biopsies. Insights gleaned from our work on murine experimental autoimmune oophoritis have been used to develop a novel ovarian biopsy strategy now under evaluation. During this period we have also demonstrated that blocking antibodies binding to human gonadotropins or gonadotropin receptors are an uncommon mechanism for premature ovarian failure. We also found that class II major histocompatibility phenotyping and testing for antibodies against the zona pellucida by indirect immunofluorescence are not useful markers for autoimmune premature ovarian failure. Intriguingly, our investigations in murine post-thymectomy polyglandular autoimmunity suggest that neonatal thymectomy causes a deficiency of CD4+ T helper 1 (TH1) activity that persists into adulthood. In ongoing work we are testing the hypothesis that this TH1-deficient state induces the autoimmune disease. Future clinical directions of the project involve development of a treatment to prevent inappropriate luteinization of follicles in patients with premature ovarian failure and a protocol to test the hypothesis that inappropriate luteinization plays a role in the infertility of maturing women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD–00634–03 DEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic Effects of insulin–like growth factor I in normal and diabetic adolescents.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	C.A. Bondy	Senior Investigator	GMS, DEB, NICHD
Others:	Mark Bach	Senior Staff Fellow	GMS, DEB, NICHD
	Edward Chin	Senior Staff Fellow	GMS, DEB, NICHD
	Rick Reinhardt	Medical Staff Fellow	GMS, DEB, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Growth and Metabolism Section

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many insulin–dependent diabetic (IDDM) adolescents have elevated growth hormone (GH) and low insulin–like growth factor–I (IGF–I) levels, which may contribute to insulin–resistance and further exacerbate already compromised mechanisms of fuel homeostasis. This study compared baseline IGF system parameters and responses to exogenous IGF–I in normal and IDDM male adolescents. Subjects were studied before and after daily 10 hour subcutaneous infusions of saline or IGF–I (20 µg/kg/hour). Baseline overnight GH secretion and IGFBP1 and 3 levels were not significantly different, but IGF–I levels were lower and IGF–II levels were higher in diabetics compared to controls. IGF–I infusion produced an increase of 3–4 fold in serum IGF–I levels and a profound reduction in IGF–II levels. IGFBP1 levels increased dramatically in diabetics and modestly in normals during two days of IGF–I infusion but IGFBP3 levels were not affected. Spontaneous overnight and arginine–stimulated GH secretion were suppressed by approximately 50% in both groups following IGF–I infusion. Insulin requirements were substantially reduced in diabetics receiving IGF–I and insulin secretion was suppressed in normals, with no evidence of a change in insulin half–life. No hypoglycemia or other adverse effect occurred during IGF–I infusions. Longer–terms trials of combined insulin/IGF–I therapy will be necessary to determine if a more physiological increase in IGF–I and normalization of GH levels can safely achieve better metabolic and anabolic balance in the treatment of IDDM in adolescents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00636-02 DEB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocrinology of Reproduction in Women

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	J.H. Segars	Sr. Clinical Investigator	UMMR, SGR, DEB, NICHD
Others:	S. Nunez	Sr. Clinical Investigator	UMMR, SGR, DEB, NICHD
	D. Arbit	NRSA Fellow	UMMR, SGR, DEB, NICHD
	G. Leong	Visiting Associate	UMMR, SGR, DEB, NICHD
	D. Rubino	Clinical Associate	UMMR, SGR, DEB, NICHD

COOPERATING UNITS *(if any)*

K. Ozato, SMGI, Laboratory of Molecular Growth Regulation, NICHD; L. Nieman, SGR, Developmental Endocrinology Branch, NICHD

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Gynecologic Research, Unit on Molecular Mechanism of Reproduction

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall objective of this project is to gain further understanding of the endocrinology of hormonal responses in women in order to provide a rational basis for the design of therapies directed toward the clinical problems of endometriosis, pregnancy loss, and fertility. The steroid hormone estrogen is central to many reproductive processes; we have therefore focused on those factors capable of modulating estrogen responsive genes in estrogen-responsive tissues. Previously, we have demonstrated that the retinoid X receptor (RXR) is capable of modulating estrogen-dependent gene activation. Advances during the current period include: 1) Demonstration that estrogen responsive genes may be specifically activated by cotransfection of the RXR and the peroxisome proliferator-activated receptor (PPAR). This activation was specific for the estrogen responsive DNA element, since mutation of the element prevented activation. Of note, estrogen responsive gene activation by these receptors occurred in the absence of the estrogen receptor. In addition, this activation was found to be augumented by addition of either 9 *cis*-retinoic acid or arachidonic acid; 2) Demonstration that the RXR and the PPAR bind specifically to an estrogen responsive DNA element in a heterodimeric complex; 3) Demonstration of several RXR-binding proteins in breast cancer cells; and 4) establishment of a method to isolate these RXR-binding proteins using an interactional cloning strategy. We plan to further characterize the role of PPAR in the activation of estrogen responsive genes. In addition, we plan to utilize an interactional cloning method to isolate and identify those proteins that may contribute to RXR modulation of estrogen responsive genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00637-01 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Steroid Hormone Action in Female Reproduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.K. Nieman	Research Medical Officer	URM, SGR, DEB, NICHD
Others:	D. Johri	Visiting Fellow	URM, SGR, DEB, NICHD
	F. Sharara	Clinical Associate	URM, SGR, DEB, NICHD
	B. Olson	Senior Staff Fellow	URM, SGR, DEB, NICHD
	T. Rarick	Clinical Associate	LTPB, NICHD
	J. Segars	Sr. Clinical Investigator	UMMR, SGR, DEB, NICHD

COOPERATING UNITS *(if any)*

Maria Merino, M.D., Clinical Pathology, NICHD

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Gynecologic Research, Unit on Reproductive Medicine

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to understand better the development of the endometrium and oocyte in women and to investigate the role of gonadal steroids in the processes. Progesterone is essential for the normal morphologic development of the luteal phase endometrium, and for maintenance of pregnancy. While relative deficiency of progesterone has been implicated in infertility and early pregnancy loss, there is no accurate non-invasive test for this. We have previously shown little correlation between endometrial maturation and serum levels of progesterone or a progestin-dependent glycoprotein, placental protein 14 (PP14). We thus evaluated the ability of exogenous hCG to stimulate consistently progesterone and PP14, so as to develop a new test for luteal function. The finding that 26 normally cycling women had progesterone levels > 11 ng/mL at 6 hours after hCG administration, suggests that this approach deserves further testing in a group of women with abnormal endometrial biopsies.

Our studies suggest that growth hormone (GH) is an important mediator of follicular development. The GH receptor is present in the granulosa cells of the developing follicle and in the corpus luteum, the estrogen and progesterone steroidogenic compartments. We also demonstrated that a complete fast for 72 hours during the mid-follicular phase does not disrupt folliculogenesis, ovulation, or menstrual cycle dynamics.

The process of uterine remodelling that is so remarkable in the mammalian uterus is achieved through a coordinated proliferation, differentiation and cell death. While the gonadal steroids estradiol and progesterone appear to be required for these organ-specific processes, the mechanism(s) by which these processes are regulated remain unclear. Potential mediators of estrogen action include IGF-1 and c-myc, a transcription factor known to be induced by growth factors, which appears to participate in the regulation of DNA synthesis in other systems. We used a well-characterized in vivo ovariectomized mouse model to correlate sex steroid-induced proliferation with the induction of IGF-1 and c-myc mRNA in the uterine endometrium. We have found that IGF-1 and c-myc levels parallel each other and the estrogen-induced processes of differentiation in this model, suggesting that they are important to proliferation. The induction of IGF-1 by GH in other tissues prompted an evaluation of whether the GH receptor was present in the endometrium, based on the hypothesis that GH might act directly on the endometrium and so modulate estrogen effect. GH receptor was found to be present throughout the endometrium, but was not regulated by estradiol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00638-01 DEB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Physiology of Hypercortisolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L.K. Nieman	Research Med Officer	URM, SGR, DEB, NICHD
Others:	J. Yanovski	Sr. Clinical Investigator	SDE, DEB, NICHD
	D. Papanicolaou	Clinical Associate	SPE, DEB, NICHD
	G.P. Chrousos	Research Med Officer	SPE, DEB, NICHD
	G.B. Cutler	Research Med Officer	SDE, DEB, NICHD

COOPERATING UNITS *(if any)*

John Doppman, M.D., Department of Radiology, CC; Edward Oldfield, M.D., SNB, NINDS; Zvi Ram, M.D., SNB, NINDS

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Gynecologic Research, Unit on Reproductive Medicine

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cushing syndrome, a fatal disease, is suspected in many thousands of patients each year, but confirmed in only a fraction of these. This project seeks to accurately identify which patients have Cushing syndrome, to define the etiology of their disease and to treat it optimally. There is no test that reliably separates individuals with pseudo–Cushing states, who have mild hypercortisolism and minimal physical features of Cushing syndrome, from those with mild or intermittent Cushing syndrome, who may present in an identical fashion. We have exploited the differences in pathophysiology of the pseudo–Cushing and Cushing syndrome states to develop new tests for this distinction. The hypercortisolism of patients with pseudo–Cushing syndrome reflects hypersecretion of CRH, suggesting that inhibition of the CRH neuron by dexamethasone, a potent glucocorticoid, would blunt the ACTH and cortisol responses to exogenous CRH. We have shown previously that patients with pseudo–Cushing syndrome have serum cortisol less than 1.4 ug/dL at 15 minutes after CRH, a criterion with 100 % specificity. We next explored whether these patients might be distinguished from patients with Cushing syndrome by inferior petrosal sinus sampling with CRH infusion, and demonstrasted that this test does not discriminate well between the groups and should not be used for this purpose.

Our second initiative, to improve the approach to the differential diagnosis of Cushing syndrome, focused on characterization of the oCRH stimulation test. This retrospective analysis of 112 patients with ACTH–dependent Cushing syndrome set criteria yielding 93% sensitivity and 100% specificity for the diagnosis of Cushing disease.

Our third aim is to improve the therapy of these patients. Recent work evaluated the efficacy of early repeat transsphenoidal exploration in the setting of persistent hypercortisolism. 12 of 17 patients had remission of hypercortisolism after a second procedure, suggesting that this approach is an alternative to radiation therapy or adrenalectomy with the advantage of low morbidity and immediacy of cure. We also examined the natural history of 10 patients with ACTH–secreting islet cell tumors. Our retrospective experience indicates that patients presenting with this etiology of Cushing syndrome have malignant disease at the time of diagnosis. Thus, this subtype of ectopic ACTH secretion appears not to be occult, and should not be sought exhaustively in the patient with an unknown source of ACTH secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD00035-22 ERRB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Structure and Function of Biologically Active Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Chen	Head, UMSPC ERRB,	NICHD
Others:	K.J.Catt	Chief	ERRB, NICHD
	J.L. Morell	Research Chemist	ERRB, NICHD
	J.H. Brown	Research Chemist	ERRB, NICHD
	L. Sun	IRTA Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Biochemical Physiology, BRMP, FCRDC, NCI, (HF. Kung)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Unit of Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the current year, this project focused on (1) the design and synthesis of peptides for immunological and structure–function relationship studies of angiotensin II type 1 (AT1) and GnRH receptor proteins; (2) design, synthesis and structure–activity relationship studies of anti–HIV peptides. AII and GnRH receptors have the seven putative domains characteristic of G protein–coupled receptors. Based on amino acid sequences deduced from cDNA encoded for AT1b, segments from both cytoplasmic and extracellular domains were selected, synthesized and conjugated to thyroglobulin or hemocyanin. In most cases, linkage of a peptide to the carrier protein was accomplished through added or existing cysteinyl residue and sulfo–SMCC cross–linker. Using six antibodies against AT1b and Western blot analysis, two species with molecular sizes Mr=66–67 kDa and 44–45 kDa were found in bovine adrenal membranes, AT1a or AT1b transfected mouse adrenal tumor cells (Y1), and rat tissue homogenates. Photoaffinity labeling of bovine adrenal membrane with ^{125}I–[Sar^{1},(4–N$_{3}$)Phe^{8}]AII followed by immunoprecipitation using the same six antisera and SDS–PAGE detected a major radioactive band corresponding 66–67 kDa, indicating it as the molecular species capable of binding the AII analogue. Similarly, seven segments with length of 12–42 residues representing various putative domains of mouse GnRH receptor cDNA deduced amino acid sequence were selected, synthesized, and conjugated to thyroglobulin for the purpose of raising antibodies against them. A peptide designated as GnRH4–42 was also synthesized for the purpose of investigating its ability to compete with the receptor for GnRH–mediated G protein coupling and PKC activation. We are synthesizing peptides covering transmembrane doamins of low α–helical propensity sequences in AII receptors and will measure their secondary structures by circular dichroism in various conditions including aqueous buffer, helicogenic solvents and phospholipid vesicles in an attempt to understand their secondary structure. The results will indicate whether a ß–sheet structure can exist in the membrane or if a different set of rules governs the secondary structure propensities in lipid environment. A series of peptides corresponding the N–terminal portion of an anti–HIV protein, GAP 31 were synthesized and analyzed for their biological activities. A 33 amino acid seqment designated as K10–K42 was found to be the shortest peptide necessary and sufficient for HIV–1 inhibition, supercoiled or viral DNA binding, viral, ribosomal or mRNA binding and ribosome inactivation. A shorter peptide (E23–K42) displayed the ribosome inactivation but not the other activites. Mapping the minimal domain of the GAP 31 offers new insights into a rational design of molecular mimetics of anti–viral drugs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD00146-19ERRB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Studies of Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.C. Chen	Head, UMSPC ERRB, NICHD	
Others:	J.L. Morell	Research Chemist	ERRB, NICHD
	J.H. Brown	Research Chemist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Department of Biochemistry, New York University School of Medicine (S. Lee-Huang)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Unit of Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the structure–function relationships, determination of amino acid sequences and identification and characterization of post–translational modifications of proteins. In continuing studies a new class of anti–HIV plant proteins, MAP 30, MAP 30II TAP 29, GAP 31, DAP 32 and 30, have been identified and characterized. These single chain ribosome–inactivating proteins inhibit HIV–1 infection as well as replication, as measured by syncytium formation, viral core protein p24 expression, and HIV–associated reverse transcriptase. Furthermore, these proteins all exhibit topoisomerase–like activity and undetectable cytotoxicity in host cells or insignificant toxicity in mice. Molecular weights of several of these proteins were determined by electrospray ionization mass spectroscopy. All were shown to be smaller than those determined by SDS–PAGE: MAP 30=29106, MAP 30II=28587, TAP 29=27168 and GAP 31=29187. Crystallization of GAP 31 was achieved in 1.6 M ammonium sulfate and 0.05 M Tris, pH 8.5 at 28°C after two weeks. The crystal diffracts up to 2.0 Å resolution and belongs to monoclinic space group $P2_1$. The cell dimensions are a=49.30(2) Å , b=44.57(2) Å , c=137.78(7) Å and β=98.32(3)°. The crystal contains two molecules of GAP 31 per asymmetric unit and 49% solvent. A detailed crystallographic study of GAP 31 by molecular replacement method using known ricin coordinates as a copy is in progress. The identification of protein kinase C phosphorylation sites in MARCKS, neurogranin, and neuromodulin allows the elucidation of dephosphorylation specificities of calcineurin and protein phosphatases 1 and 2A. Co–localization of these phosphatases and PKC substrates and high concentration of calcineurin are consistent with the notion of interacting–regulatory actions among protein phosphatases, protein kinases, and calmodulin. The amino acid sequence analysis of fragments upon proteolysis of a number of prohormone peptides by yeast aspartic protease 3 (YAP3) permitted determination of specificity of the protease. YAP3 cleaved proinsulin at the paired–basic amino acid residue site of the B–C chain junction as well as the C–A chain junction. This protease generated Leu–enkephalin and Leu–enkephalin–Arg from dynorphin A (1–11) and dynorphin B (1–13) respectively, indicating cleavages at a paired–basic residue site motif which has an Arg or Lys at the downstream P4' position, respectively. Porcine cholecystokinin 33 was cleaved at two mono–basic amino acid residue sites at Lys^{23} and Arg^{9} which contain an upstream Arg at the P6 and P5 position, respectively. These results show that YAP3 recognizes three different motifs: paired–basic amino acid residues sites containing an additional basic residue at the upstream P4–P6 or downstream P4'–P6' positions.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00147-18-ERRB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Action of Peptide Hormones in Steroidogenic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.L. Dufau	Chief, SME	ERRB, NICHD
Others:	Y.-C. Koh	Visiting Associate	ERRB, NICHD
	Y. Miyagawa	Visiting Fellow	ERRB, NICHD
	E. Buckzo	Adjunct Scientist	ERRB, NICHD
	A. Fabbri	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Molecular Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytochrome P450-17α (CYP17), the key enzyme for the synthesis of androgens and glucocorticoids, catalyses two consecutive oxidation reactions to produce 17α-hydroxylated (hydroxylase) intermediate and androstene products (17-20 lyase). Within the conserved domain (343 to 363), charged amino acids contribute to lyase and hydroxylase activities. Arg 346 is essential for lyase activity and Arg 363 is of importance for hydroxylase activity. Genomic analysis revealed that this domain is contained in exon 6. Substitution of Arg361 and Glu358 within the conserved domain with alanine abolished all hydroxylase and lyase activities. These mutants showed a pattern of premature degradation in transfection studies. Although the mutant protein levels were close to wild type at early times, the mutant protein was inactive at these time periods. Pulse chase studies revealed marked decreases in half-life from 10 hours to 2 hours. Additional substitutions show that a negative charge and the extended length of the side chain of Glu358 is of importance for the hydroxylase and lyase activities. Our studies indicate that Glu358 and Arg 361 may contribute to the folding of the steroid binding domain of CYP17 and that their substitution results in misfolding and degradation.

In the Leydig cells, serotonin is an acute stimulus of CRF secretion and its action is exerted via high-affinity low capacity 5HT2 receptors that are located in the plasma membrame. In recent studies we defined the regulation of serotonin secretion in cultured Leydig cells. Adult Leydig cells secrete considerable quantities of serotonin. The release was acutely stimulated by human chorionic gonadotropin and by cyclic adenosine monophosphate, indicating its major role in gonadotropin-induced serotonin release. hCG stimulation did not require extracellular calcium. However, ionomycin was a potent stimulus of serotonin release, indicating that increases in cytoplasmic calcium could also induce amine release. The serotonin content of Leydig cells decreased during stimulation of serotonin release. Immunocytochemical studies revealed specific staining of serotonin in the interstitial cells of the adult rat testis. Testicular serotonin has local antireproductive actions and may seve as the trigger of an autocrine microcircuit that buffers the gonadotropin stimulus. Intratesticular increases of serotonin concentration could occur in conditions known to increase testicular (varicocele) and central (stress) serotonin, and may contribute to the disruption of testicular function observed in these states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00150-19-ERRB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Gonadal Receptors and Gonadotropin Biological Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Maria L. Dufau	Chief, SME	ERRB, NICHD
Others:	C.-H. Tsai-Morris	Senior Staff Fellow	ERRB, NICHD
	E. Buckzo	Adjunct Scientist	ERRB, NICHD
	Z.Z. Hu	IRTA Fellow	ERRB, NICHD
	L. Zhuang	Adjunct Scientist	ERRB, NICHD
	Y. Geng	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions HD-6-2904
University of Virginia, Department of Medicine, Charlottesville, VA (J. Veldhuis)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As an SP1-driven Tataless promoter domain was localized within the 173 bp 5' flanking region of luteinizing hormone receptor gene. Within this region cis-elements and transbinding factors responsible for stimulatory/inhibitory control of the gene and tissue-specific expression were identified. Transcription is dependent on the presence of two SP1 elements (Sp1$_2$ and Sp$_4$) that contribute equally to transcript initiation. Promoter activity is regulated by at least three additional DNA domains [R domain (-1266 to -1307 bp), C-box (-42 to -73 bp, and M1 (-24 to -42 bp)] that bind multiple transfactors in a tissue-specific manner. Basal promoter activity is dependent on a functional M1 domain in LH receptor-expressing mouse Leydig tumor cells (mLTC) but not in non-expressing CHO cells. The C-box binding factors either inhibit promoter activity or block inhibition through overlapping but non-identical DNA binding domains (AP-2 and NF-1 elements). Removal of the AP-2 element in the C-box results in mLTC-specific transcriptional activation that may involve an mLTC M1/C-box interaction. In addition, competition for C-box factors by an upstream regulatory element that is only inhibitory in CHO cells indicates that both the C-box inhibitory AP-2 protein and the C-box neutral NF-1 proteins compete for this upstream domain in a tissue-specific manner. Competition between the inhibitory and neutral DNA binding factors within both upstream and promoter domain could provide a mechanism for the control of LH receptor gene expression in gonadal cells. Within the 6.2 kb of the 3'non-coding region of the gene, two functional pA domains (H1 and H2) produce two sets of major mRNA transcripts of 2.6/2.3 kb encoding the holoreceptor and alternatively spliced B form, respectively, and the corresponding high MW (5.8 and 5.5) mRNAs for both forms. The functional efficiency of each pA domains is related to the intrinsic nature of the pA signals and is enhanced 5-7 fold in the presence of downstream genomic sequence of up to 2.6 kb 3' to H1, or 1.3 kb 3' to H2; it is also influenced by tissue-specific factors. An insertion of the functional pA domain (H1) and the known trasposon R domain (a repetitive DNA line R domain) in the LHR gene is postulated to have occurred following divergence from the TSHR and FSHR genes in evolution.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00184-16 EBBR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Pituitary Hormone Secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K.J. Catt	Head, SHR	ERRB, NICHD
Other:	K. Arora	Sr. Staff Fellow	ERRB, NICHD
	L. Krsmanovic	Adjunct Scientist	ERRB, NICHD
	D. Spergel	NSF Fellow	ERRB, NICHD
	J. Reinhart	IRTA Fellow	ERRB, NICHD
	W.-Y. Paik	NIH Intl. Fellow	ERRB, NICHD
	S.-H. Lee	Adjunct Scientist	ERRB, NICHD
	D. Mason	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS (if any)

Philipps Universitat Marburg, Marburg, Germany (O. Ortmann, G. Emons)
Lab of Cell Biology and Genetics, NIDDK (E. Rojas, M. Kukuljan)
Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

GnRH receptors are expressed in native and immortalized (GT1) GnRH-producing neurons and influence their intrinsic pattern of pulsatile neuropeptide secretion by an autocrine feedback mechanism. GT1 cells also expresses receptors for glutamate and GABA, transmitters that influence GnRH release from the hypothalamus *in vivo*. In GT1 cells, glutamate acts via NMDA and AMPA/kainate receptors to stimulate calcium influx and subsequently GnRH secretion. GnRH itself stimulated the expression of c-fos in GT1 cells. These findings indicate that glutamate can act directly on GnRH neurons and could promote GnRH release in the hypothalamus without activation of intermediate neurons. The increased expression of c-fos that occurs in GnRH neurons *in vivo* during the proestrous surge of gonadotropin could be stimulated by a positive feedback action of GnRH on its neuronal receptors. In pituitary gonadotrophs, the short-term stimulatory and long-term inhibitory effects of progesterone on GnRH-stimulated LH were found to be accompanied by significant changes in the calcium signaling responses to agonist stimulation, with stimulatory or inhibitory actions according to the duration of progesterone treatment. Also, the primary increase in phospholipase C-mediated phosphoinositide hydrolysis was followed by activation of phospholipase D that was mediated by protein kinase C, and led to a large secondary increase in diacylglycerol production. Thus, GnRH activates a dual phospholipase pathway in a sequential and synchronized manner that prolongs the signaling response to agonist simulation. In studies on the angiotensin II (AT1) receptor, the cloning of an amphibian AT1 receptor that recognizes peptide but not non-peptide antagonists was utilized to define the receptor binding site for losartan by constructing mutant mammalian receptors in which non-conserved amino acids were replaced by the corresponding amphibian residues. Specific amino acid residues located in five of the seven transmembrane domains of the receptor were found to be involved in the binding of the non-peptide antagonist, but not the peptide antagonist. In addition, the relationship between receptor activation and internalization was examined in a series of mutant receptors with modifications in the third cytoplasmic loop and/or at a critical residue for signal transduction (Asp74) in the second transmembrane domain. This work demonstrated that endocytosis of the agonist-AT1 receptor complex is independent of agonist-activated signal transduction and second messenger formation, and indicated that receptor internalization and activation of phospholipase C have different conformational requirements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01HD00187-15-ERRB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormonal Regulation of Cellular Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.-P. Huang	Head, SMR	ERRB, NICHD
Others:	F. Huang	Research Chemist	ERRB, NICHD
	C.W. Mahoney	Senior Staff Fellow	ERRB, NICHD
	K. Seki	Visiting Fellow	ERRB, NICHD
	L.Shen	Visiting Fellow	ERRB, NICHD
	J. J. Miloszewska	Visiting Fellow	ERRB,NICHD

COOPERATING UNITS *(if any)*

Department of Biophysics, Kanazama Univ. School of Medicine, Japan (H. Higashida); Lab of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH (P.M. Blumberg)

LAB/BRANCH

Endocrinology and Reproductive Research Branch

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	6	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C (PKC) has emerged as a pivotal regulatory element for the regulation of many cellular functions. Multiple PKC subspecies have been identified by molecular cloning and the various isozymes were found to have distinct tissue, cellular, and subcellular distributions and were differentially expressed during development. The functional roles of these kinases in the control of cellular differentiation were tested with N1E115 neuroblastoma cells. PKC-activating phorbol ester counteracts the cAMP-induced neurite outgrowth of these cells by selective translocation and activation of PKC βI but not PKCζ. Activation of PKC βI results in the phosphorylation of a M_r=90k protein, whereas activation of cAMP-dependent protein kinase attenuates its phosphorylation. A method for preparing substrates specific for a group of PKC isozymes has been devised based on onteraction of cationic polypeptide with Ca^{2+}/phospholipid/detergent mixed micelles to form aggregates with a unique surface structure. Neurogranin (Ng), neuromodulin (Nm), and MARCKS are the most prominent PKC substrates in the brain. Ng and Nm bind calmodulin (CaM) with high affinity in the absence of Ca^{2+}, whereas MARCKS does so in the presence of Ca^{2+}. Phosphorylation of these proteins by PKC reduces their affinities toward CaM and thus release CaM for the CaM-dependent enzymes. In addition to the phosphorylation-induced effects, oxidation of Ng by peroxide or nitric oxide also reduces the affinity of this protein for CaM. Ng and Nm are dephosphorylated by protein phosphatase 1, 2A, and calcineurin and MARCKS is more effectively by protein phosphatase 1 compared to phosphatase 2A and calcineurin. These latter two phosphatases selectively recognize a set of sites in MARCKS, whereas phosphatase 1 dephosphorylates all the sites. Binding of phosphoinositides to MARCKS inhibits the phosphorylation of this protein. The phosphorylated MARCKS has a reduced affinity toward these phospholipids and may contribute to the translocation of this protein from membrane to cytosol upon stimulation of PKC. A novel M_r=28k PKC/casein kinase II substrate has been identified and its cDNA cloned. This protein has been shown to be a substrate of casein kinase II in N18 neuroblastoma cells. The genomic structures of the CNS-specific PKC γ and Ng were analyzed for the purpose of defining tissue-specific and development-regulated expression of these two genes. The promoter activity of the Ng gene can be stimulated by phorbol ester or by co-transfection with PKC cDNAs. A M_r=20k protein binding to the AT-rich regions of both PKC γ and Ng gene promoters has been identified and phosphorylation of this protein by PKC attenuates its binding to DNA elements containing ATTA, ATAA and AATA motifs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00193-09 ERRB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Angiotensin II Receptors and Activation Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K.J. Catt	Head, SHR	ERRB, NICHD
Others:	T. Balla	Visiting Scientist	ERRB, NICHD
	A.J. Baukal	Chemist GS-12	ERRB, NICHD
	K. Sandberg	Sr. Staff Fellow	ERRB, NICHD
	H. Ji	Visiting Fellow	ERRB, NICHD
	L. Hunyady	Adjunct Scientist	ERRB, NICHD
	Y. Tian	Adjunct Scientist	ERRB, NICHD
	S. Nakanishi	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*

Department of Physiology, Semmelweis University Medical School, Budapest

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In studies on the angiotensin II (AT1) receptor, the cloning of an amphibian AT1 receptor that recognizes peptide but not non-peptide antagonists was utilized to define the receptor binding site for the clinically effective AII antagonist, losartan, by constructing mutant mammalian receptors in which non-conserved amino acids were replaced by the corresponding amphibian residues. Specific amino acid residues located in five of the seven transmembrane domains of the receptor were found to be involved in the binding of the non-peptide antagonist, but not the peptide antagonist. In addition, the relationship between receptor activation and internalization was examined in a series of mutant receptors with modifications in the third cytoplasmic loop and/or at a critical residue for signal transduction (Asp74) in the second transmembrane domain. This work demonstrated that endocytosis of the agonist-AT1 receptor complex is independent of agonist-activated signal transduction and second messenger formation, and indicated that receptor internalization and activation of phospholipase C have different conformational requirements. During analysis of AT1 receptor-activated signaling pathways in adrenal glomerulosa cells, the myosin light chain inhibitor wortmannin was found to prevent the sustained but not the initial inositol phosphate/calcium (InsP3/Ca2+ response to agonist stimulation. This was shown to result from a decrease in InsP3 formation and secondary reduction of Ca2+ entry through the plasma membrane. Other structurally unrelated inhibitors of myosin light chain kinase also inhibited AII-induced InsP3/Ca2+ signaling, demonstrating that wortmannin and functionally similar compounds act primarily on the formation of InsP3 at a step that is sensitive to inhibition of myosin light chain kinase or a similar enzyme. In related work on the Ca2+-dependent sustained phase of the agonist-induced InsP3 response, this important second component of the intracellular signaling mechanism was shown to be inhibited by several inorganic Ca2+ channel blockers. In addition, the Ca2+ surrogate Sr2+ was partially active in sustaining Ins(1,4,5)P3 production in cells stimulated with AII in Ca2+-free medium. Sr2+ was found to support the activity of phospholipase C but not of Ins(1,4,5)P3 3-kinase, the enzyme responsible for conversion of Ins(1,4,5)P3 to Ins(1,3,4,5)P4, and to lead to an accumulation of the former in agonist-stimulated cells. Thus, Sr2+ can potentiate agonist-induced elevations of Ins(1,4,5)P3 in the intact cell, and provides a means for direct analysis of the inositol tris-/tetrakisphosphate pathway in cellular signaling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00194-06ERRB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Steroid Biosynthesis and Metabolism in the Mammalian Adrenal Cortex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.A. Strott	Head, SSR	ERRB, NICHD
	Y.C. Lee	Chemist	ERRB, NICHD
	W.J. Driscoll	Senior Staff Fellow	ERRB, NICHD
	K. Komatsu	Visiting Fellow	ERRB, NICHD
	K. V. Venkatachalam	IRTA Fellow	ERRB, NICHD
	H. Chiba	Special Volunteer	ERRB, NICHD
	N. Luu	Pre-IRTA Fellow	ERRB, NICHD

COOPERATING UNITS *(if any)*
Armed Forces Radiological Research Institute (Dr. Mark Whitnall)
Clinical Neurosciences Branch, NIMH (Dr. Brian Martin)
Unit on Molecular Structure and Protein Chemistry, NICHD (Dr. Hao-Chia Chen)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Steroid Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biotransformation of steroids by conjugation to highly charged chemical groups such as glucosiduronidate and sulfonate is a process of immense importance that results in the conversion of these essentially hydrophobic compounds to a more water soluble state. While the glucosiduronidation of steroids is considered preparatory to their ultimate excretion, the biological import of steroid sulfonation remains a poorly understood. Sulfonation, the transfer of a sulfonate group from a universal donor to an appropriate acceptor site, is carried out by enzymes termed sulfotransferases. There are multiple biochemical and functional implications of steroid sulfonation. For instance, by increasing steroid polarity and water solubility, sulfonation can influence steroid transportability or function as a steroid trapping and storage mechanism. By acting as either an initiating event (steroid sulfonate is the active form) or a terminating event (steroid sulfonate is the inactive form), sulfonation can function to regulate steroid physiologic responsivity. An interesting and challenging conundrum is the fact that one of the most active tissues to sulfonate steroids is the adrenal cortex. Thus, in an effort to shed light on the biological consequences of steroid sulfonation in the mammalian adrenal cortex, the SSR is engaged in the isolation, cloning and transcriptional regulation of adrenocortical steroid sulfotransferases. Three steroid sulfotransferases, namely estrogen sulfotransferase (EST) and two stereoselective 3-hydrosysteroid sulfotransferases (3a-HST/3b-HST) are the current focus. Molecular cloning and expression of EST and 3a-HST have been completed; the cloning of 3b-HST is in progress. The structural basis for the exquisite stereospecificity of the HSTs will be examined by sequence analysis, mutational studies and chimeric constructs. The 5'-flanking region of the EST gene has been cloned and the complete structural gene is now being determined. EST is a unique protein of multiple charge isoforms that demonstrate distinct catalytic and high affinity steroid binding activities. It is of particular interest that EST has a nuclear localization and that EST and the HSTs are differentially expressed in functionally distinct adrenocortical zones. The binding site for the activated sulfonate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) has been identified and the precise amino acid residues involved in the PAPS-protein interaction have been determined by mutational studies. Enzymes responsible for the synthesis of PAPS are being purified and cloned. Additionally, a PAPS-specific sulfatase is undergoing purification for biochemical analysis and cloning.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00195-01 ERRB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Intracellular signaling in endocrine cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S.S. Stojilkovic	Head, UCS	ERRB, NICHD
OTHERS:	M. Tomic	Visiting Fellow	ERRB, NICHD
	L. Zheng	Visiting Fellow	ERRB, NICHD
	M. Cesnjaj	Adjunct Scientist	ERRB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Cell Biology and Genetics, NIDDKD (E. Rojas)
Mathematical Research Branch, NIDDK (Y.-X Li and J. Rinzel)
Bone Research Branch, NIDR (F. Luyten and S. Vukicevic)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Unit on Cellular Signaling

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms by which G protein-coupled and tyrosine kinase receptors control calcium signaling, and the physiological implications of such signaling, were investigated in several cell types. Three patterns of calcium signaling were observed: base-line oscillatory, with the frequency but not the amplitude of spiking controlled by agonist concentration; slow-oscillatory, with a constant frequency and variable amplitude of spiking; and non-oscillatory. The pattern of calcium signaling was not determined by the receptor subtype but by post-receptor events. Base-line calcium oscillations were initiated by injection of inositol trisphosphate (InsP3), as well as by exposure to compounds such as ionomycin, thapsigargin, and thimerosal. However, when the cytoplasmic oscillator was activated by these agents, it operated only at the basal rate of 5/min and its spiking frequency did not change with increasing drug concentrations, as commonly occurs in agonist-and InsP3-stimulated cells. In contrast, both types of oscillations were affected by the depletion of intra-luminal calcium and by changes in [Ca2+]i, but were not inhibited by ryanodine. The voltage-sensitive calcium entry pathway also affected InsP3-dependent calcium oscillations in excitable gonadotrophs. In agonist- and InsP3-stimulated cells, sustained calcium oscillations were extinguished by hyperpolarization after 3-15 min despite the availability of calcium in the extracellular medium. Single depolarizing pulses transiently restored the amplitude of the sustained spiking in a dihydropyridine- and extracellular calcium-sensitive manner. The responses to depolarization showed a marked dependence on membrane potential that was correlated with the steady-state inward calcium current. In addition, the repetitive application of brief depolarizing pulses modulated the frequency of agonist and InsP3-controlled spiking. These extrinsically driven and extracellular calcium-dependent oscillations were sensitive to the calcium-pump blocker, thapsigargin, but not to ryanodine. A mathematical model based on these experimental observations gave responses to a wide range of agonist doses, including the subthreshold responses, superthreshold base-line oscillatory response with frequency determined by [InsP3], biphasic oscillatory, and biphasic non-oscillatory response. The model also predicted the existence of non-receptor-mediated calcium oscillations. Calcium signaling is sufficient to trigger the expression of primary response genes (PRGs) in several types of endocrine cells. In pituitary gonadotrophs, the protein kinase C-dependent induction of PRGs was found to be modulated both positively and negatively by physiological changes in [Ca2+]i. Thus, the pattern of calcium signaling may represent an efficient mechanism for the control of gene expression in endocrine and other cell types.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00131-20 HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Biochemical Genetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William A. Gahl, M.D., Ph.D., Head, HGB, NICHD
Others: I. Bernardini, Chemist, HGB, NICHD
S. Kaler, M.D., Medical Staff Fellow, HGB, NICHD
D. Krasnewich, M.D., Ph.D., Medical Staff Fellow, HGB, NICHD
S. Hahn, M.D., Medical Staff Fellow, HGB, NICHD
L. Gallo, M.D., Medical Staff Fellow, HGB, NICHD
G. Holt, Ph.D., Senior Staff Fellow, HGB, NICHD
G. McDowell, Ph.D., IRTA Fellow, HGB, NICHD
P. Ye, Ph.D., IRTA Fellow, HGB, NICHD

COOPERATING UNITS *(if any)*

See Attached

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Human Biochemical Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892-1830

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.2	6.2	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Members of the Section continue to perform linkage analysis on several families with nephropathic cystinosis. To date, 75% of the human genome has been excluded as the location of the cystinosis gene.

2. Several mutations in the Menkes disease gene have been identified in patients with this X-linked disorder. Four members of a family with mild Menkes disease displayed an A to T transversion in the +3 splice donor site of an intron near the 3' end of the Menkes gene, resulting in deletion of a 118 bp exon, with substantial correct splicing. One patient with occipital horn syndrome had an A to G transition in the -2 exonic splice donor site in the middle of the Menkes gene resulting in deletion of a 92 bp exon, again with residual normal splicing. Two related patients with classical Menkes disease exhibited a G to T transversion at a -1 exonic splice donor site causing a glutamine to histidine substitution at codon 724, skipping of one, two, or three exons, and premature termination.

3. Analysis of N-linked oligosaccharides on serum glycoproteins from patients with Carbohydrate Deficient Glycoprotein Syndrome indicates a defect early in the formation of dolichol-oligosaccharides.

4. Mutational analysis of the fumarylacetoacetase gene in a 12-year old boy with chronic tyrosinemia type I revealed two defects, a missense mutation causing a tryptophan to glycine alteration in codon 234, and a splicing mutation in the +5 position of intron 12 causing exon skipping.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 00137-20 HGB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation and Expression of the UDP-Glucuronosyltransferase Gene Family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ida S. Owens	Head	HGB, NICHD
Others:	Marco Ciotti	Visiting Fellow	HGB, NICHD
	Jeong Cho	Visiting Fellow	HGB, NICHD
	Nahid Gholamai	Visiting Fellow	HGB, NICHD
	Theresa Huang	Technician	HGB, NICHD
	Christine Potter	Technician	HGB, NICHD

COOPERATING UNITS *(if any)*
Frederick Cancer Research Facility, Frederick, MD (Ronald Lubet).
Imperial College of Science, Technology, and Medicine, London, England (Dr. D.R. O'Reilly)
University of Rochester, Department of Pharmacology, (Dr. J. P. Jones).

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Genetic Disorders of Drug Metabolism

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.3	3.3	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our description of the *UGT*1 locus has proven critical for the determination of genetic defects in patients with Crigler-Najjar(CN) diseases. The original version of the gene complex (*UGT1A-UGT1F*) codes for at least 2 bilirubin (Br), 3 Br-like, and 1 phenol transferases. In the 5' region, six different exons 1, each with an upstream promoter and each encoding the amino terminus of an isoform, are arrayed in series with 4 common exons encoding six identical carboxyl termini in the 3' region of the locus. The most 5'exon was used to select other cosmid clones to extend the locus by more than 125 kb. At least five additional exons 1 in three cosmid clones have been identified, mapped for restriction endonucleases, and analyzed by Southern hybridization. Two of the exons have been sequenced and arranged in the series. The newly identified exons are related to the unique region of a second phenol, HLUG P4, reported in the literature. We have isolated and sequenced the corresponding cDNA for one of the newly characterized exons 1, and the cDNA has been expressed in order to determine substrate specificity. Predictably, a critical mutation in a unique exon affects a single form, whereas one in a common exon inactivates the entire locus. Both the deletion of a Phe-170 and a G276R missense mutation in exon 1A of the *UGT1A* gene of separate CN Type I patients affect the amino terminus of the HUG-Br1 isoform. The code in patient 1 abolished a critical di-phenylalanine in a conserved hydrophobic Micro-region A (MR A, codons 161-180) which according to computer-determined secondary structure is critical to the formation of a buried hydrophobic helical structure, a structure not seen in all other isoforms (which lack the di-phenylalanine) examined. Also two conserved Pro in the MR A are required for activity. All mutations in MR A generated pH-sensitive activity; there was complete loss of activity at the newly uncovered major pH optimum of 6.4, but normal activity at the routinely used pH 7.6 assay condition. In patient 2 a missense mutation, G276R, disrupted a strictly conserved di-glycine (codons 276/277) in a conserved Micro-region B (MR B, codons 270-288). Mutations in MR B indicate that of two conserved Pro, the 170, but not 287, is required for activity; also conserved Val-275 is not required. For MR B loss of activity occurred at both pH 6.4 and 7.6. Evidence shows that the di-glycine is contained in a β-hair pin loop and is present in all transferase isoforms despite its coding by a unique exon 1 at the *UGT1* locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00404-12 HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. DeBrohun Butler, Ph.D.	Senior Investigator	HGB, NICHD
Others:	E. McIver	Biology Aid	HGB, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Developmental and Metabolic Neurology, NINCDS, Laboratory of Cell Biology and Genetics, NIDDK, Section on Developmental Genetics, Human Genetics Branch, NICHD, Laboratory of Cellular and Developmental Biology, NIDDK

LAB/BRANCH

Human Genetics Branch

SECTION

Unit on Genetic Diseases Involving Cystine Storage

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892-1830

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Commercially obtained cystine binding protein (CBP) of E. coli has been purified by HPLC, yielding a pure cystine binding protein not similar to any protein in the data banks. This protein has been partially sequenced, an N-terminal peptide made and an antibody to this peptide obtained which reacts with the whole protein. The commercial preparation of CBP also contains a histidine binding protein which can be used to assay for histidine in the same way that CBP is used to assay tissues for cystine. CBP of E. coli is being cloned by two methods. One, by using an E. coli expression library and identifying the correct CBP producing colony with our antibody to CBP. Two, by making oligonucleotides of sections of the known N-terminal sequence of CBP and using them as primers to identify CBP in the E. coli library.

Niemann-Pick C disease (NPC) was shown to be reversibly induced in normal fibroblasts by hydrophobic amines and certain steroids, thus creating a model system for study of this disease. We are identifying the trafficking patterns and metabolism of intracellular cholesterol using various inhibitors and stimulators.

We have shown that normal and cystinotic fibroblasts take up ascorbic acid by two mechanisms, a high affinity Na+ dependent transporter and a low affinity Na+ independent transporter, neither of which can be accounted for by diffusion. Transport of ascorbic acid by the low affinity transporter is not different from normal in the two types of cells, however transport in cystinotic cells by the high affinity transporter is about 50% of that seen in normal cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01HD00408-11HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Heritable Disorders of Connective Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Joan C. Marini, M.D., Ph.D.	Senior Investigator	HGB, NICHD
Others:	Qin Wang, M.D.	Fogarty Fellow	HGB, NICHD
	Piotr Wieczorek, Ph.D.	Fogarty Fellow	HGB,NICHD
	Adriana Sarafova, Ph.D.	PSC	HGB, NICHD
	Diane Trybul	Biologist	Ebon Contract

COOPERATING UNITS *(if any)*

Department of Rehabilitation Medicine, CC (N.L. Gerber); Laboratory of Developmental Endocrinology (G. Chrousos); Sect on Metabolic Analysis and Mass Spectrometry (A. Yergey); Genetics Unit, Shriners' Hospital, Montreal (F. Glorieux)

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Connective Tissue Disorders

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892-1830

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	3	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Section has continued its studies aimed at elucidating the molecular mechanisms of heritable disorders of connective tissue, specifically osteogenesis imperfecta (OI) and Ehlers-Danlos (EDS), and at applying this information to the treatment of these disorders.

One primary interest of the Section is to identify the collagen mutations in patients with OI and EDS and determine the relationship between the type and location of the mutation and the severity of the connective tissue disorder. Mutations in the $\alpha2(I)$ collagen chain identified by this Section and other labs have provided additional support for the regional model we have proposed.

A second primary interest of the Section is to develop selective antisense suppression of the mutant collagen allele as an approach for therapeutic intervention. We have used thio oligos targeted to mutant mRNA and nuclear RNA in the cultured fibroblasts of a patient with type IV OI. We have achieved selective suppression of the mutant allele and are pursuing approaches such as ribozymes and vector constructs to increase suppression efficiency.

A third focus of interest which we have been developing is in the bone biology of OI. We are using cultured osteoblasts to study the way bone cells modify and secrete mutant collagen. We are also pursing the secondary non-collagenous abnormalities of OI matrix.

In clinical studies, we are continuing our treatment trial of growth hormone in short children with OI to determine its effects on growth stimulation, bone density and bone morphometric properties. We are continuing our collaborative interests in the neurological aspects of OI and in maximizing the physical functioning of OI children though aggressive rehabilitation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD00414-05 HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurogenetics and Neural Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Lawrence Charnas, M.D., Ph.D.	Senior Staff Fellow	HGB, NICHD
Others:	Christine Potter	Biologist	HGB, NICHD
	Steven Scott	Junior Fellow	HGB, NICHD
	Dalano Hoang	Stay in School	HGB, NICHD

COOPERATING UNITS *(if any)*

Department of Rehabilitation Medicine, CC (U. A. Chaudhry); OD and MNB, NINDS (M. Dalakas, C. Luciano) Clinical Branch, NEI (M. Kaiser-Kupfer), HHMI, University of Pennsylvania (R.L. Nussbaum), National Naval Medical Center (R.C. Miller), Johns Hopkins Medical Institutions (T. A. Brushart), CPB, NIMH (H. Leonard, A. J. Allen),

LAB/BRANCH

Human Genetics Branch

SECTION

Human Biochemical Genetics

INSTITUTE AND LOCATION

NICHD

TOTAL STAFF YEARS: 2.5	PROFESSIONAL: 1	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00415-04 HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiology and Molecular Biology of Arachidonate Metabolism and CFTR Mutation in CF

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anil B. Mukherjee, Ph.D., M.D.	Head	HGB, NICHD
Others:	Peter Yuan, Ph.D.	Visiting Fellow	HGB, NICHD
	Gopal C. Kundu, Ph.D.	IRTA Fellow	HGB, NICHD

COOPERATING UNITS *(if any)*

University of Pittsburg
Bureau of Biologics, FDA
University of California, San Diego

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Developmental Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892-1830

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.25	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cystic fibrosis (CF) is caused by mutations in the CF transmembrane regulator (CFTR) gene. The deletion of Phe 508 (ΔF_{508}), in CFTR is the most common (70%) mutation in CF. One of the major complications of CF is the development of severe inflammation of the airways of these patients. We have previously demonstrated that the epithelial cells derived from CF patients who carry ΔF_{508} mutation in CFTR release abnormal levels of arachidonic acid when stimulated by agonists. It is not clearly understood how an abnormality in CFTR may cause an abnormality in arachidonate metabolism. There is compelling evidence to suggest that cells carrying the ΔF_{508} mutation in the homozygous state have altered acidification of intracellular organelles and defective c-AMP-induced membrane recycling. Last year we reported that the regulation of phospholipase A_2 (PLA_2)-catalyzed arachidonic acid release may be defective in CF lymphocytes and fibroblasts. We demonstrated that calcium ionophore A23187-induced arachidonic acid (AA) release in the following cell lines: T84 cells (a colon carcinoma line which expresses high level of normal CFTR; CFPAC-1 cells (a pancreatic carcinoma line from a ΔF_{508} CF patient) and clones derived from CFPAC-1 cells after transfection with a retroviral vector containing the normal CFTR gene (CFPAC-PLJ-CFTR) or with vector only (CFPAC-PLJ). These data indicated that the cell lines which contained a mutant CFTR in their genome and had a defective c-AMP-induced Cl^- efflux appeared to have 5-10 fold higher AA release than those in control cells. This increased release is dependent upon extracellular Ca^{++} and is further enhanced by phorbol-12-myristate, 13-acetate(PMA, stimulator of PKC) but not by the inactive derivative 4α phorbol,12,13 didecanoae and is inhibited by staurosporine (a PKC inhibitor). An inhibitor of PLA_2, quinacrine (200μM) abolished AA release in these cells. These data suggested that Ca^{++} and pKC-stimulated AA release, possibly catalyzed by PLA_2, is dysregulated in cells carrying the ΔF_{508} CFTR mutation in CF. These data indicated that an alteration of a Ca^{++}, PKC-regulated AA release raising the possibility that this abnormality may be due to activation of $cPLA_2$. We now report that $cPLA_2$ is not involved in the abnormal release of AA from these cells. It appears that a Ca^{++}-independent PLA_2 may be involved in this process. Transcription of several PLA_2s in the mutant as well as control cells are now being studied to determine the type of PLA_2 that may dysregulated in CF as a result of ΔF_{508} mutation. A clear understanding of the mechanism of AA release may allow development of rational therapeutic approaches to control inflammation in the airway of these CF patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00417-04 HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Basis of α1-Antitrypsin Deficiency

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Mark Brantly, MD.	Senior Staff Fellow	HGB, NICHD
Others:	J. H. Lee, MD.	Visiting Fellow	HGB, NICHD
	N. Novoradovskaya, Ph.D.	Visiting Fellow	HGB, NICHD
	V. De Gouvea, Ph.D.	Special Volunteer	HGB, NICHD
	S. Ong, MS.	Biologist (Ebon)	HGB, NICHD
	D. Hoang	Special Volunteer	HGB, NICHD

COOPERATING UNITS *(if any)*

See attached

LAB/BRANCH

Human Genetics Branch

SECTION

Unit on Genetic Disorders of Secreted Proteins

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892-1830

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.25	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our studies have focused on determining the molecular basis of α1-antitrypsin (α1AT) deficiency variants and utilizing natural mutations of the α1AT gene to assess structure-function relationships of α1AT and to explore regulatory mechanisms of α1AT gene expression. In the past year the laboratory has begun characterization of an α1AT intronic sequence that enhances α1AT gene expression, characterized the molecular basis of QOtrastevere (an α1AT null variant), begun the characterization of a group of frameshift mutations that terminate in the same structural region of the α1AT protein and identified a new null variant QObonny blue. We have developed an adenoviral-α1AT vector that expresses six times more α1AT than previous vectors and established an emphysema animal model to evaluate the biochemical efficacy of α1AT gene therapy. To evaluate the hypothesis that the liver disease associated with α1AT deficiency is the result of hepatitis from a common enteric virus we screened liver samples from α1AT deficient individuals post transplant for the presence of viral nucleic acid sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00910-15 HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Uteroglobin, cc10kDa protein, Phospholipase A_2, Osteopontin and HIV-1 Protease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anil B. Mukherjee, M.D., Ph.D.	Head	HGB, NICHD
Other:	Alessandro Peri, M.D.	Visiting Fellow	HGB, NICHD
	Gopal C. Kundu, Ph.D.	IRTA Fellow	HGB, NICHD
	Gary Zhang, M.D., Ph.D.	IRTA Fellow	HGB, NICHD
	Peter Yuan, Ph.D.	Visiting Fellow	HGB, NICHD
	Ijeoma Nwaeze	Stay-in-School	HGB, NICHD

COOPERATING UNITS *(if any)*

University of Naples, Italy, University of Rome, Georgetown University, George Washington University McGill University, Harvard Medical School, University of Pittsburgh, Naval Research Laboratory, Washington Hospital Center, Medlantic Research Foundation

LAB/BRANCH

Human Genetics

SECTION

Developmental Genetics

INSTITUTE AND LOCATION

NICHD, Bethesda, Maryland 20892-1830

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have:(a) demonstrated that Clara cell 10kDa (cc10kDa) protein is the counterpart of rabbit uteroglobin (rUG) by obtaining over expression of recombinant cc10kDa in *E.coli* and detailed characterization of this protein; (b) established that human UG (hUG) gene is expressed in many organs and tissues beside the pulmonary Clara cells suggesting that hUG may be a multifunctional cytokine-like protein; delineated that hUG gene expression in the human endometrium is regulated by ovarian steroid hormones; (d) established and characterized two temperature-sensitive epithelial cell lines derived from the rabbit endometrium which differentiate *in vitro* and express UG gene in response to steroid hormones; (e) developed a highly improved expression vector which stabilizes the cDNA to be expressed in a bacterial host and requires no ampicillin for this stability; (f) crystallized recombinant hUG and carried out X-ray diffraction studies; (g) carried out multidimensional NMR studies on hUG which suggest a structure for this protein which is nearly identical to rUG; (h) site-directed mutagenesis of hUG suggests that mutation of Lys-43-> Asp 43 leads to inactivation of phospholipase A_2-inhibitory property of hUG; (i) discovered a novel high affinity cell surface binding protein for hUG on NIH 3T3 and human trophoblast cells and when huG binds to this putative receptor it inhibits chemoinvasion of the artifiicial basement membrane by these cells. In addition, the choriocarcinoma cells were found to be lacking this receptor and their invasion is not affected by hUG; (j) established that invasion of prostatic carcinoma cells is suppressed by hUG; (k) identified a specific region of group I PLA_2(residues 21-40) interaction of which with a neutralizing antibody leads to complete inhibition of its activity; (l) filed an invention report on the anti-invasive effects of hUG (m) established and standardized a radiometric assay for HIV-1 protease;(n) completed a preliminary study on the beneficial effects of hUG on a primate model of neonatal respiratory distress syndrome. The results suggest that recombinant hUG inhibits lung PLA_2 activity and prevents migration of inflammatory cells into this organ. Long-term effects of this therapy are now being investigated (o) established that osteopontin (OP)-fibronectin interaction in bone and extracellular matrix (ECM) protein is mediated by transglutaminase (TG) and (p) demonstrated that disruption of cellular Src (c-Src) protooncogene causes dramatic suppression of OP gene expression. Since OP is essential for new bone formation by ostcoclas, our results may in part explain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00912-15 HGB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Regulation and Cellular Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Janice Y. Chou, Ph.D.	Head	HGB, NICHD
Others:	K-J. Lei, Ph.D.	Visiting Scientist	HGB, NICHD
	M. Chamberlin, Ph.D.	NRC Fellow	HGB, NICHD
	C. Park, Ph.D.	Visiting Fellow	HGB, NICHD
	T. Ubagai, M.D.,	Visiting Fellow	HGB, NICHD
	See attached		

COOPERATING UNITS *(if any)*

German Cancer Research Center (Drs. G Schutz and S Ruppert); University of Florida (Dr. C-L Chen); Georgetown University (Dr. Y-W Chan); Duke University (Dr. Y-T Chen); Baylor University (Dr. AL Beaudet); University of North Dakota (Dr. R Nordlie)

LAB/BRANCH

Human Genetics Branch

SECTION

Cellular Differentiation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892-1830

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.0	5	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glycogen storage disease (GSD) type 1 is an autosomal recessive disease caused by the deficiency of glucose-6-phosphatase (G6Pase). Five GSD type 1 subgroups exist which correspond to defects in the G6Pase catalytic unit (1a), a stabilizing protein (1aSP), the glucose-6-P (1b), phosphate/pyrophosphate (1c), and glucose (1d) translocases. We have characterized the cDNA and gene for G6Pase, analyzed the *G6Pase* gene of twelve type 1a patients, and uncovered a total of six mutations that cause GSD type 1a. We have also characterized the *G6Pase* gene of GSD type 1b, 1c, and 1aSP patients. Our results show that the *G6Pase* gene of GSD type 1b and 1c patients is normal. However, an R83C mutation found in several GSD type 1a patients was identified in both *G6Pase* alleles of the type 1aSP patient, indicating that type 1aSP is a misclassification of GSD type 1a.

Hepatic S-adenosylmethionine (AdoMet) synthetase catalyzes the biosynthesis of AdoMet which functions in transmethylation and transsulfuration. Deficiency of hepatic AdoMet synthetase causes persistent hypermethioninemia and unusual breath odor. To understand the molecular basis of this disorder, we characterized the cDNA and gene for human hepatic AdoMet synthetase. Using SSCP and DNA sequencing analyses, we identified four mutations in the *AdoMet synthetase* gene of two compound heterozygous patients.

To understand the molecular mechanisms underlying differential *PSG* expression, we characterized promoter elements of six *PSG* genes. Nucleotides -172 to -34 with respect to the translation start site constitute a minimal promoter in class 1 PSG genes and only nucleotides -172 to -80 are necessary for promoter activity in class 2 PSG genes. Class 2 genes contain a perfect Sp1 recognition sequence which is necessary for promoter activity and binds to an Sp1-like molecule in placental extracts. Moreover, an activator sequences is located within nucleotides -83 to -34 in *PSG12* (a class 1 gene) which binds to a 50 kDa protein share immunological reactivity with the transcription factor PEA3.

We have demonstrated that the 5'-flanking region of a rat *PSG* gene, *rnCGM3*, contains two promoter elements (PI and PII) located upstream and downstream of the transcription start site, respectively. The PISII site within the PI promoter binds to C/EBPβ in placental extracts and transactivates *rnCGM3* expression. C/EBPβ, a leucine zipper family of transcription factor, is expressed in high levels in the placenta and constitutes one of the transcription factors that positively regulate *rnCGM3* expression during pregnancy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 0054-20 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Behavioral Analysis of Vocal Communication in Squirrel Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen J. Suomi	Chief, LCE	LCE, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01106-11 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Continuity of Individual Differences in Reactivity in Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S. J. Suomi	Head	LCE, NICHD
K. L. Rasmussen	Senior Staff Fellow	LCE, NICHD
M. Champoux	Staff Fellow	LCE, NICHD
J. D. Higley	Staff Fellow	LCS, NIAAA
G. D. Byrne	IRTA Fellow	LCE, NICHD
D. Smucny	Pre-IRTA Fellow	LCE, NICHD
S. Higley	Research Technician	LCE, NICHD

COOPERATING UNITS *(if any)*

NIAAA (Linnoila, Goldman); NIMH (Doudet); Delta Reg. Primate Ctr. (Clarke); Primate Lab., U of Wisconsin (Schneider); U of Puerto Rico (Phoebus); U of Colorado (Laudenslager); SUNY - Buffalo (Berman); U of California, S.F. (Boyce); U of Minnesota (Gunnar)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.3	4.0	1.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates primate biobehavioral development through comparative longitudinal studies of rhesus and capuchin monkeys, with special emphasis on characterizing individual patterns of differential behavioral and physiological responses to environmental novelty and challenge and on determining long-term developmental consequences for individuals of different genetic backgrounds reared in different physical and social environments. During the past year extensive neonatal testing of rhesus monkey infants reared either by their biological mothers or with peers revealed heritable influences on activity state profiles independent of rearing condition and rearing influences on CSF concentrations of the serotonin metabolite 5-HIAA independent of pedigree within the infants' first month of life; these differences were, in turn, predictive of differential behavioral, adrenocortical, and monoamine reactivity to environmental challenge at 6 months and beyond. An interaction between biobehavioral reactivity and chronic level of environmental stress was found to be predictive of injury rates among members of a free-ranging rhesus monkey troop: high-reactive monkeys had lower injury rates than low-reactive monkeys under low stress conditions, but significantly higher injury rates than their low-reactive counterparts under high stress conditions. Thus, high reactivity appears to be a protective factor in the absence of chronic stress but paradoxically becomes a significant risk factor for injury under conditions of high environmental stress. A comparison of heartrate patterns among wild-living adult female rhesus monkeys revealed strong familial influences, with closely related females showing significantly greater concordance of heartrate parameters than distantly related females, who in turn showed greater concordance than nonkin. A separate study of captive group-living female rhesus monkeys demonstrated that low CSF levels of 5-HIAA were associated with low social dominance, poor social competence, and a high incidence of impulsive aggression, replicating associations previously reported for both captive and wild-living rhesus monkeys males. Finally, major methodological advances were achieved in biological sampling techniques, telemetric monitoring of physiological functioning in free-ranging monkeys, and primate PET-scan brain imaging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01107-11 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adaptation of Laboratory Reared Monkeys to Field Environments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

S. J. Suomi	Head	LCE, NICHD
A. Timme	Fogarty Fellow	LCE, NICHD
G. Westergaard	IRTA Fellow	LCE, NICHD
P. O. Wagner	Research Psychologist	LCE, NICHD
C. Price	Biologist	LCE, NICHD
M. Jorgensen	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

OACU (Bayne); Dept. of Psychology, U. of Massachusetts (Novak); Dept. of Psychology, Georgia State U. (Hopkins); U. of Florida (Boinski); Istituto di Psicologia, CNR (Visalberghi)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	2.5	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates how rhesus monkeys and other nonhuman primate species born and raised under different laboratory conditions adapt to placement into naturalistic outdoor environments that contain specific physical and social features of the monkeys' natural habitat. Adaptation is ~~-~~assessed by examining behavioral repertoires and by monitoring a variety of physiological systems in these subjects, yielding broad-based indices of relative physical and psychological well-being. The responses of subjects to experimental manipulations of selected features of their respective environments are also assessed in similar fashion. During FY94 construction of a new addition to the shelter facility for the LCE's 5-acre outdoor enclosure at the NIHAC was begun, curtailing some of the ongoing research with the resident rhesus monkey troop. However, data collection for a series of studies investigating spacial memory capabilities regarding the location of familiar and novel objects within the 5-acre enclosure was completed, and the effects of the physical and social disruption produced by the construction on ongoing troop behavior and habitat usage was documented. Lifespan longitudinal behavioral profiles exhibited by a comparison group of rhesus monkeys maintained from birth in indoor housing demonstrated remarkable stability of both individual and gender-specific profiles from early adulthood to senescence, despite significant developmental changes in certain behaviors as a function of increasing age. Extensive studies investigating tool-using behavior in capuchin monkeys greatly expanded the known range and complexity of tool-using tasks that the species is capable of mastering, including certain tasks previously thought to be limited to humans and their direct hominid ancestors. Another set of studies employed video game technology to demonstrate impressive cognitive capabilities involving elements of self-recognition in capuchin monkeys qualitatively similar to those of chimpanzees and humans. Data from several field studies investigating the relationship between group social structure and context and acoustical features of vocalizations in 3 species of New World primates were published this past year. Finally, major methodological advances were achieved in the editing and analysis of psychophysiological data collected via telemetry from free-ranging monkeys, as well as in the development of sophisticated growth curve modelling techniques for analyzing small-n longitudinal data sets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01108-10 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparative Studies of Play Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen J. Suomi	Chief, LCE	LCE, NICHD

COOPERATING UNITS *(if any)*

LAB.BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01112-08-LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Home and Out-of-Home Care on Child Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	H. Wessels	Visiting Fellow	LCE, NICHD
	K.J. Sternberg	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*

Center for Human Growth and Development, U of Michigan (Bookstein); Institute of Psychology, University of Goteborg (Broberg, Hwang).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves a longitudinal study conducted in Sweden examining the effects of center day care, family day care, and home care on the development of 145 children recruited at an average of 16 months of age. Multivariate analyses have consistently indicated that type of care has no reliable impact on child development, but that the quality of home care and the quality of alternative care have substantial effects on the children's verbal abilities, their social skills, and their behavioral attitudes. The children were most recently assessed when they averaged 9 years of age and were completing their second year of formal schooling. To date, data on the development of verbal and mathematical abilities as well as the development of personal maturity (as measured by field-independence, ego-control, and ego-resilience) have been analyzed in relation to the varying child-care histories the children have experienced since their first enrollment in the study. These analyses indicate that family daycare has modest but significant negative effects on both verbal abilities and personal maturity, whereas center-based care has beneficial effects in both domains. The effects of the quality of home and alternative care diminish as children move into the formal educational system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 01113-08 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael E. Lamb	Head, SSES	LCE, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01114-07 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Individual Differences in Physical and Affective Functioning in Infancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	B. Leyendecker	Fogarty Fellow	LCE, NICHD
	A. Scholmerich	Fogarty Fellow	LCE, NICHD
	M. T. Estrada	Pre-IRTA Fellow	LCE, NICHD
	L. Shelley	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)* University of Osnabruck, Germany (H. Keller); Institute for Applied Socialization Research, Berlin, Germany (L. Ahnert); Department of Anthropology, Washington State University (B. Hewlett); Department of Human Development, U. of Maryland (S. Porges); Institute of Psychological Research, University of Costa Rica (D. Miranda); L. Baradaran; G. Bernal; P. Brown; M. P. Fracasso; C. Larson

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	2.2	2.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on long-term consequences of individual differences in physical, social, and affective functioning and the description of early interaction in diverse ecological contexts. In one longitudinal study, measures of behavioral synchrony in the home predicted differences in the security of infant-mother attachment as reported by the mothers. In a second project, we are studying infant-mother interaction and attachment in infants from upper-middle class Euro-Americans and low-class Central American families, developing culturally-sensitive criteria for evaluating normative social behavior and development. Regardless of social or ethnic background, mothers spent comparable amounts of time in discrete activities such as feeding, caring for, and playing with their infants. Comparable observations of mothers and infants in Costa Rica are designed to further explore the effects of culture and context on early interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01115-07 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Domestic Violence and Evaluation of Children's Testimony

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	K. J. Sternberg	Research Psychol	LCE, NICHD
	K. Brittin	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

Hebrew U, Jerusalem (Greenbaum); Jerusalem Municipality (Garber, Lorey, Cialik); U of Rochester (Cicchetti); Ministry of Social Welfare, Jerusalem (Hovav, Karniel, Eden, Berger); USAF (Gerns, Larison, Mollerstrom); P.W. Esplin; I. Hershkovitz; T. Manor; Y. Orbach; A. Redlich, L. Yudilevitch.

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This program of research involves two research projects. The goal of the first project is to explore the effects of domestic violence on children who were either victims of physical abuse, witnesses of spousal abuse, both victims and witnesses, or neither victims nor witnesses. These children were first assessed in 1988/9 when they were between 8 and 12 years of age and the results of these assessments have been reported in previous annual reports. The children, their parents, their teachers, and their schools are now being reinterviewed. These assessments will be completed in FY 95 and analyses of the data will then begin.

The goal of the second study is to assess the field reliability and validity of a system for eliciting and assessing statements by children who are alleged to have witnessed sexual abuse. Sites around the United States and in Israel are providing transcripts of audio- or videotaped interviews along with case files to establish case facts independent of the statements. The statements are being assessed using a revision of the Criterion-Based Content Analysis (CBCA) techniques, while case facts are independently rated using "ground truth" assessment scales developed by SSED staff. Other analyses are focused on the relationship between interviewer style and quality of information provided by the child. Several studies have confirmed that open-ended questions elicit longer and more detailed responses than more focused questions, regardless of the number of incidents experienced and the language (English or Hebrew) in which the interview was conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01116-07 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Social Development Across the Lifespan in Diverse Cultures and Ecologies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. E. Lamb	Head, SSED	LCE, NICHD
Other:	K. J. Sternberg	Research Psychologist	LCE, NICHD
	C. Bassen	NRSA Fellow	LCE, NICHD
	S. Dawud Noursi	Pre-IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

U. of NC, Greensboro (MacKinnon); U. of MD, Baltimore (Serpell); Ecole Normale, Cameroon (Nsamenang); Hebrew University, Jerusalem (Greenbaum).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	1.6	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The work on this project involves a number of studies in a variety of cultures. The overall objective is to explore the ways in which developmental environments can be described by variations in physical and social ecologies, especially in terms of parental beliefs, values, and practices, and to assess how differences in these domains affect children's development. In one study, Palestinian mothers, grandmothers, and great-grandmothers are being interviewed about their perceptions, values, expectations, responsibilities, and practices. The goal is to explore transgenerational changes in childrearing practices and beliefs in a culture exposed to tremendous exogenous influences, especially over the last half century. In a second study, researchers are attempting to assess specific maternal and child attributions about one another in order to identify the extent to which attributions or expectations shape the way that parents and children interact, with particular focus on the development of aggressive behavior. Mothers and sons interacted more aggressively when they had negative attributions of one another. In the third study, gender differences in the self-perceptions of young adolescents are being assessed so that staff can explore the antecedents and correlates of different styles of self-perception.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01117-07 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Hospitalization Experience: Children's Coping with the Stress of Surgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. H. Bornstein	Head	LCE, NICHD
	J. L. Genevro	NRC Biotech Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH Laboratory of Comparative Ethology

SECTION Section on Child and Family Research

INSTITUTE AND LOCATION NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research ~~project~~ examines age-related differences in children's understanding of and reactions to a brief stay in the hospital for elective surgery. The research integrates the adult stress and coping literature with research on changes in children's cognitive capabilities as they mature, as well as with the behavioral pediatrics literature pertaining to preparing children for hospitalization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01119-07 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Specificity of Mother-Infant Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head, CFRS	LCE, NICHD
J. Suwalsky	Research Psychologist	LCE, NICHD
K. Painter	Research Psychologist	LCE, NICHD
M. Fivel	Research Psychologist	LCE, NICHD
C. Andreassen	IRTA Fellow	LCE, NICHD
C. Rahn	Research Psychologist	LCE, NICHD
M. Haynes	Statistician	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	1.7	2.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates endogenous and exogenous factors that contribute to the development of cognitive and social competencies in children during the first four years of life. Before children are old enough to enter formal social learning situations, nearly all of their experiences stem directly from interactions they have with their primary caregivers. The present study focuses on the extent to which several maternal characteristics (age, parenthood status, SES, parity, employment status as well as type of substitute care experienced during mother's employment) influence observed relations between caregiving on the one hand and child developing cognitive and social competencies on the other. In this longitudinal study, subjects are visited when they are 5, 20, and 48 months of age. At 5 months, mother-infant interaction is observed. At 20 months, measures of toddler functioning (play competence, language development, and social adaptation) and maternal behavior (play and I.Q.) are obtained. At 48 months, the follow-up examines diverse children's abilities (representational competence, problem solving, reasoning skills, mathematical ability, language use and discourse, and selected aspects of generally adaptive behavior).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 HD 01120-07 LCE

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Parenting and Infant Activity: A Multiculture Perspective

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head, CFRS	LCE, NICHD
O. M. Haynes	Statistician	LCE, NICHD
C. Rahn	Research Psychologist	LCE, NICHD

COOPERATING UNITS *(if any)*
ARGENTINA (deGalperin and Berti); BELGIUM (Vyt); FRANCE (Pêcheux); ISRAEL (Maital); ITALY (Venuti and Lis); JAPAN (Azuma, Ogino, and Toda)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiences in infancy are acknowledged to affect the course and outcome of social and mental development in childhood, and they are credited for some of the distinctiveness of culture. More specifically, cross-cultural developmental studies have historically shown that variations in childrearing styles typically have implications for children's later cognitive performance and interpersonal behavior. Home environments are thought to reflect larger cultural values, beliefs, and customs, and many social theorists have contended that the family generally, and the mother-infant relationship specifically, may be vital to development of the individual and basic to the organization of the culture. As a result, investigators have frequently studied infancy and mother-infant interaction in attempts to address questions about the origins and development of cultural identity. Of course, each society has evolved patterns of childrearing adjusted to its own special demands. For example, it is widely held that Japanese and Americans differ in prominent aspects of their psychological make-ups and that certain social and intellectual distinctions between members of these two cultures arise early in life. Previous study on the nature of infant development in Israeli kibbutzim determined that many decisive aspects of infant care -- particularly the close ties between infant and mother -- vary markedly from the typical American experience. Contemporary France, Italy, and America are relatively similar in terms of industrial level, educational attainment, and living standards, yet these societies differ considerably in terms of history, sociology, and culture. Within-culture comparisons are equally important: Studies of development in Belgium offer answers to the question of how being reared in monolingual versus bilingual environments affects cognition, communication, and socioemotional development. Argentina contrasts middle-class with extremely poor rearing conditions, all in a South American setting virtually unresearched. What differences exist in parenting and in infant activity within and across these cultures? A central purpose of this project is to identify similarities and differences in the childrearing ecologies of Argentine, Belgian, French, Israeli, Italian, Japanese, and U.S. American infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01122-07 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Longitudinal Assessment of Children's Mental and Social Abilities

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head, CFRS	LCE, NICHD

COOPERATING UNITS *(if any)*

Infancy Studies Program, New York University (Tamis-LeMonda)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research continues a longitudinal study of mental and social development in two cohorts of children observed repeatedly between 2 months and 4 years of age. The premise of this research is that learning in childhood occurs primarily within an interpersonal context; thus, a central goal is to describe how diverse infant and maternal behaviors relate to the ontogeny of central dimensions of children's mental and social competencies. Children participating in the assessment constitute longitudinal cohorts seen at 2, 5, 13, 20, and 48 months of age. Major objectives of this research are to study stability, continuity, and interaction in mental and social development over the first years of life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01123-04 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Correlates and Neural Mechanisms of the Infant Cry and Related Vocalizations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.D. Newman	Head, DNS,	LCE, NICHD
Others:	K. Moody	IRTA Fellow	LCE, NICHD
	J. Norcross	IRTA Predoctoral Fellow	LCE, NICHD
	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

Cooperating Units: Department of Zoology, University of Maryland at College Park (C.S. Carter); Laboratory of Clinical Science, NIMH (T. Insel); Wisconsin Regional Primate Research Center (D. Abbott)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Developmental Neuroethology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Immunocytochemical techniques were applied for the first time in the common marmoset to locate the intracerebral sources of the neuropeptides oxytocin and vasopressin. Both vasopressin and oxytocin reactivity was found in cells within the paraventricular and supraoptic nuclei of the hypothalamus in adult males and females.

2. Under the influence of the anticholinergic drug benactyzine hydrochloride, castrated male squirrel monkeys exhibited alarm reactions of greater intensity than intact males, actually exceeding the reactivity levels of intact females.

3. Correlations between endogenous hormone levels and social behavior were found in newly paired common marmosets. Luteinizing hormone levels were positively correlated with incidence of a territorial marking vocalization, the twitter, in females. Scent marking, a nonvocal component of territorial marking, was negatively correlated with cortisol levels in both males and females.

4. A study of social cognition in adult squirrel monkeys demonstrated the ability to discriminate between familiar and unfamiliar individuals presented as videotaped images.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01124-04 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic and Experiential Influences on the Development of Primate Vocal Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.D. Newman	Head, SDN	LCE, NICHD
Others:	K. Moody	IRTA Fellow	LCE, NICHD
	J. Norcross	Predoctoral IRTA Fellow	LCE, NICHD
	D. Bernhards	Bio. Lab. Tech.	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Developmental Neuroethology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Juvenile common marmosets with no prior exposure to neonates were tested at varying intervals up to 4 weeks after the birth of neonates into their family group. Retrieval latencies averaged around 40 seconds to both familiar and unfamiliar infants in the same juveniles who had completely failed to show retrieval behavior when tested with unfamiliar infants prior to this experience. As little as 60 hours of exposure to neonates was sufficient for retrieval behavior to be expressed. Thus, the data from this study strongly indicate that caregiving is an active process promoted by stimuli arising from the presence of a neonate in the family group. The stimulus role for the neonate appears to be as important in adults as in juveniles and subadults.

Study of vocal development in rhesus macaques indicated that differential rearing (nursery with peers vs mother with mixed age group members) may influence the rate of maturation of vocal signalling, and that, in mother-reared infants, males and females develop at different rates with respect to some aspects of vocal behavior.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01125-02 LCE

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Psychophysiological Substrates of Cognitive Processing and Socioemotional Expression from 2 to 36 Months

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M. H. Bornstein	Head, CFRS	LCE, NICHD
S. A. Linnemeyer	IRTA Fellow	LCE, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This longitudinal study approaches child development through the analysis of autonomic function, cognitive processing, and socioemotional expression at 2, 5, 13, 20, and 36 months in healthy term infants. In relation to these areas, specific behaviors of the mother (autonomic, intellectual, and interactional) are examined. A combination of physiological assessment, psychometric measurement, and observational techniques is utilized to examine comprehensively the roles of cognitive processing and socioemotional expression in early child development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00707-10-LCMN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacological Studies of Synaptic Transmission In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. L. Mayer	Visiting Scientist	LCMN, NICHD	12 months
Others:	Morris Benveniste	Senior Staff Fellow	LCMN, NICHD	6 months
	Kathryn Partin	NRC Fellow	LCMN, NICHD	12 months
	Doris Patneau	Senior Staff Fellow	LCMN, NICHD	9 months
	Derek Bowie	Visiting Fellow	LCMN, NICHD	5 months
	Christine Winters	Chemist	LCMN, NICHD	12 months

COOPERATING UNITS *(if any)*

Dr. A. Buonanno, LDN, NICHD, NIH, Professor J. C. Watkins, Dept. Pharmacology, University of Bristol, United Kingdom

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Section of Neurophysiology and Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	3.7	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This section investigates functional properties of excitatory amino acid receptors in the vertebrate CNS, utilizing electrophysiological and molecular biological techniques. Fast perfusion systems are used for concentration jump application of glutamate receptor selective agonists and antagonists to cells and membrane patches under voltage clamp. Preparations in use include primary cultures of hippocampal neurons and glial cells, and recombinant receptors expressed in transfected cells and oocytes. For NMDA receptors we have used agonist trapping during open channel block by 9-AA as a novel approach to estimate maximum open probability. Analysis of the molecular basis of AMPA receptor modulation by cyclothiazide is being investigated based on our observation that alternative splicing of the flip and flop variants, generated by use of alternative exons encoding 37 highly conserved amino acids, but which differ in core regions of only 4 + 1 amino acids, generates receptors with high and low sensitivity to cyclothiazide. Assay of cyclothiazide action on recombinant receptors, in which theses core residues are being exchanged, is being used to determine critical sites for allosteric modulation.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01205-02-LCMN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular and Synaptic Physiology of Hippocampal Interneurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. J. McBain	Senior Staff Fellow	NICHD, LCMN	12 months
Others:	Lei Zhang	Visiting Fellow	NICHD, LCMN	10 months
	Gianmaria Maccaferri	Guest Researcher	NICHD, LCMN	8 months
	Jing Du	Visiting Fellow	NICHD, LCMN	4 months

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Unit on Cellular and Synaptic Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to characterize the properties of voltage gated channels and synaptic transmission of hippocampal inhibitory neurons in the developing brain and how these properties impact hippocampal function under both physiological and pathological conditions. Although much is known about their neurochemistry, their role in the local circuits and the basic electrophysiological properties of inhibitory interneurons, little is known about the specific ionic or ligand gated channels expressed on this highly divergent population of cells. A major part of our effort is to understand the ionic mechanisms which regulate the activity of these cells and how these mechanisms impact hippocampal function. It is hoped that these mechanisms may be potentially exploited in the future treatment of a variety of pathological conditions such as electrographic seizure activity, ischemic cell damage and stroke.

Our work over the past year has focused on a particular population of inhibitory neurons, the CA1 stratum oriens/alveus interneuron. Particularly we have begun to investigate and characterize the compliment of potassium (K) channels present on these cells using both an electrophysiological and a molecular approach. In addition we have begun to characterize the role of these stratum oriens/alveus interneurons during the plastic phenomena of long term depression and potentiation. The role of stratum oriens/alveus interneurons in the high K^+ -induced electrographic seizure model of epilepsy has also been studied. The characterization of the basic physiological and pharmacological properties of these cells is an essential first step for the more detailed study proposed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 1206-01 LCMN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

RECEPTOR MEDIATED CALCIUM SIGNALLING IN GLIA AND NEURONS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Dr. James Russell	Head, Section on Neuronal Secretory Systems	LCMN, NICHD	12 months
Others:	Ms. L. A. Holtzclaw	Biologist	LCMN, NICHD	12 months
	Dr. C. A. Sheppard	NRC Fellow	LCMN, NICHD	12 months
	Dr. P. Holbrook	Senior Staff fellow	LCMN, NICHD	12 months
	Dr. S. Yagodin	Visiting Scientist	LCMN, NICHD	12 months

COOPERATING UNITS *(if any)*

Dr. V. Gallo	Head, Unit on Neurobiology	LCMN, NICHD
Dr. D. C. Klein	Head, Section on Neuroendocrinology	LDN, NICHD

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Section on Neuronal Secretory Systems

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Section aims to understand the principles, and mechanisms that govern intracellular calcium signalling in glial cells and neurons. Although neurons are the central players in cellular communication and information processing in the brain, recent discoveries of glial cell types, excitability and signalling properties have shown that glial cells may play an important role in long distance signalling in the brain. Although glial cells do not show electrically excitable properties similar to neurons, they do possess a form calcium based excitability in response to activation of neurotransmitter receptors. Furthermore, most physiological processes are regulated by receptor mediated signal transduction mechanisms and in a vast majority of cases these mechanisms utilize cellular calcium signals as triggers for physiological processes. Such calcium signals have recently been recognized to have complex temporal and spatial characteristics. Oscillatory [Ca2+]i changes which propagate as calcium waves and highly localized [Ca2+]i signals have been recorded in many cells. The research focus of the Section includes studies on the signal transduction mechanisms of various receptor systems as well as the cell biology of calcium signalling in both glial cells and neurons. The objectives are to understand cellular processes that control signal transduction mechanisms for Ca2+ signalling and cellular processes that regulate temporal and spatial signalling, at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HD 02000-03-LCMN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurotransmitter Receptors in GLIA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Vittorio Gallo	Visiting Scientist	LCMN, NICHD	12 months
Others:	Montse Molne	Visiting Fellow	LCMN, NICHD	12months
	Steve Scherer	IRTA Fellow	LCMN, NICHD	12 months
	Mario Pende	Guest Worker	Italian NRC Fellow	12 months
	Paul Wright	Senior Lab Technician	Ebon Contractor	12 months

COOPERATING UNITS *(if any)*

Dr. R. Armstrong, Dept. Anatomy & Cell Biology, USUHS; Dr. C. Szpirer, Dept. de Biologie Moleculaire Universite Libre de Bruxelles, Belgium; Dr. M. Rocchi, Universita de Bari, Italy; Drs. N. Jenkins and N. Copeland, NCI, FCRDC.

LAB/BRANCH

Laboratory of Cellular and Molecular Neurophysiology

SECTION

Unit on Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	6/12	12/12

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular and functional properties of neurotransmitter ligand-gated ion channels in glial cells were studied, in order to understand their regulation and function in the brain. Studies were carried out to: A. characterize the physiological and molecular properties of glutamate-gated ion channels in glia and B. to clone a glutamate receptor gene and analyze its 5' regulatory region in order to understand its transcriptional regulation in glia.

A. Physiological and molecular properties. Primary cultures of rat oligodendrocyte progenitors (O-2A) and the primary cell line CG-4, derived from rat O-2A cells, were used as a model system. CG-4 and primary O-2A cells develop to oligodendrocytes *in vitro* similarly to O-2A progenitors *in vivo*. In both types of cultures, the intermediate filament protein nestin, highly expressed in neuroepithelial cells *in vivo*, is found at the O-2A stage, but down-regulated in differentiated oligodendrocytes. A similar downregulation of nestin expression was also demonstrated in oligodendrocytes *in vivo*. Kainate- and AMPA-preferring glutamate receptors are co-expressed in cells of the oligodendrocyte lineage. Intracellular Ca^{2+} transients, due to transmembrane influx of the divalent cations, and immediate early gene (*NFI-A* and *c-fos*) transcription are regulated by glutamate receptors in cells of the oligodendrocyte lineage. Genes encoding kainate-preferring glutamate receptor subunits are expressed very early in neural development (embryonic day 10 rat embryos) and in undifferentiated, nestin-positive multipotential precursors of the rat neural tube. B. Glutamate receptor genes and analysis of their 5' regulatory region. Genes encoding kainate receptor subunits are highly expressed in cells of the oligodendrocyte lineage, as determined by Northern blot and RT-PCR analysis of total RNA. The cloning of one of these subunit genes (*GRIK5*, which encodes the kainate-preferring subunit KA2) was accomplished. Two kb of the 5' regulatory region of the rat *GRIK5* were sequenced and analyzed, as well as their transcriptional potential in oligodendrocyte progenitors of the CG-4 cell line. The chromosomal and subchromosomal localization of *GRIK4* (encoding the kainate preferring subunit KA1) and *GRIK5* was determined in mouse, rat and human by interspecific backcross mapping, Southern analysis of cell hybrid panels and fluorescence *in situ* hybridization. In all three species, *GRIK4* and *GRIK5* were found to map on separate chromosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01301-12 LDMI

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Human Immune Response to Polysaccharide-Protein Conjugate Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Schneerson	Research Medical Officer	LDMI, NICHD

See Attached

COOPERATING UNITS *(if any)* LCDB, NIDDK (J. Shiloach); SUNY Downstate Medical Center, N.Y. (G. Schiffman); Carolinas Medical Center, Charlotte, N.C., (J.C. Parke, Jr.); National University Hospital, Iceland (J. Jonsdottir), LCI, NIAID (J. Bennett); [illegible]

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892-2720

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.8	4.3	3.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The surface polysaccharides of bacterial pathogens, which include capsular polysaccharides and lipopolysaccharides, serve as protective antigens. The immunologic properties of these bacterial polysaccharides, namely their age-related and T-cell independent immunogenicity, limit their use as vaccines. Covalently attachment to medically-useful proteins to form conjugates, both increases their immunogenicity and confers T-cell dependent properties to these polysaccharides. The capsular polysaccharides of Streptococcus pneumococcus types 6B and 14, Staphylococcus aureus types 5 and 8, Group B streptococcus type 3, and Escherichia coli K1 have been bound to several proteins and their immunologic properties assayed in mice. The pneumococcal type 6B and Group B streptococcal conjugates have been evaluated clinically. The safety of conjugate-induced antibodies reactive with E. coli K1 was evaluated in an infant rat model. The LPS of shigellae was detoxified, their O-specific polysaccharides bound to bacterial toxoids and their immunogennicity in mice found to be satisfactory. In Phase 1 and Phase 2 studies, these conjugates of the O-specific polysaccharides were safe and immunogenic: LPS antibody levels elicited by the investigational conjugates were similar to those in recruits convalescent from shigellosis. In preliminary studies, a S. sonnei-rEPA conjugate protected against shigellosis caused by this pathogen. More extensive trials of its clinical efficacy are underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01304-12 LDMI

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protective Effect of LPS Antibodies Against Shigella

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.B. Robbins	Chief	LDMI, NICHD

COOPERATING UNITS *(if any)*
Pasteur Merieux Serums & Vaccins, France (D. Schulz); WRAIR, Washington, D.C. (D. Taylor); Israeli Defense Force, Israel (D. Cohen); Sheba Medical Center, Israel (J. Passwell); LMC, NIDDK (C.P.J. Glaudemans); LCDB, NIDDK (J. Shiloach).

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892-2720

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01308-12 LDMI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conjugate-Induced Polysaccharide Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.C. Szu Research Chemist LDMI, NICHD

See Attached

COOPERATING UNITS *(if any)* Walter Reed Army of Research, Washington, DC (D.N. Taylor, J. Sadoff); LMC, NIDDK (C.P.J. Glaudemans, P. Kovac); LCDB, NIDDK (J. Shiloach)

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Unit on Molecular Biophysics and Biochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892-2720

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.7	4.4	2.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evidence continues to accrue that vaccine-induced serum antibodies to the surface polysaccharides of enteric bacteria confer protective immunity against these pathogens. To improve the immunologic properties (and hence the efficacy) of the Vi, which is now a world-licensed vaccine for typhoid fever, this polysaccharide was covalently bound to bacterial toxoids to form a conjugate vaccine. Phase 1 studies confirmed the superior immunity of a conjugate using the recombinant exoprotein A of _Pseudomonas aeruginosa_ (_r_EPA). Negotiations for Phase 2 and Phase 3 studies with this investigational vaccine are underway in Vietnam, an area with a high attack rate of typhoid fever especially in children. The structure, physical chemical and immunologic properties of a pectin, whose repeat unit of α-_D_-(1->4)GalA is identical to the backbone of Vi, was modified by treatment with acetic acid dihydrazide. The resultant di-_O_-acetylated derivative was antigenically indistinguishable from Vi. A conjugate, composed of a di-_O_-acetylated pectin as a vaccine for typhoid fever, is being prepared for clinical evaluation. _Escherichia coli_ O157, considered as an important emerging pathogen in the U.S. and throughout the world, resembles closely shigellae especially _Shigella dysenteriae_ type 1. The O-specific polysaccharide was purified and covalently bound to the exotoxin of _Clostridium welchii_ C (pig bel toxin) and _r_EPA. The conjugates elicited IgM and IgG antibodies in mice which were bactericidal. Phase 1 studies with these investigational vaccines are approved and will soon be initiated. The serologic response of adult volunteers elicited by a cholera O-specific polysaccharide-_r_EPA conjugate is being completed. The conjugate elicited higher IgG LPS antibodies that the cellular vaccine. The IgG LPS antibodies were bactericidal and more extensive studies with this investigational vaccine are being planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01311-01 LDMI

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Polysaccharide Vaccine for Mycobacterium tuberculosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.B. Robbins Chief LDMI, NICHD

See Attached

COOPERATING UNITS *(if any)* LCDB, NIDDK (J. Shiloach); CBER, FDA (S. Morris, J. Muller, A. Karpas); Montefiore Hospital, Bronx, NY (B. Curry); Statens Seruminstitut, Denmark (A. Anderssen); Pasteur-Mérieux Serum & Vaccins, France (D. Schulz)

LAB/BRANCH

Laboratory of Developmental and Molecular Immunity

SECTION

Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892-2720

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	4.3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Worldwide, tuberculosis remains a serious cause of morbidity and mortality: in the U.S., emergence of multi-antibiotic-resistant strains is probably due to the rapid passage of the pathogen in patients with AIDS. Although it is the most frequently used vaccine in the world, there is no good evidence that BCG prevents primary pulmonary tuberculosis. Our research program to develop a vaccine for M. tuberculosis is based upon its similarity to capsulated bacterial pathogens that is derived from experimental data in the literature: 1) serum antibody-mediated opsonphagocytosis by peripheral blood polymorphonuclear leukocytes; 2) the presence of a capsular polysaccharide (denoted as electron translucent zone or ETZ); 3) similarity between the age incidence of tuberculous meningitis and that due to capsulated pathogens; and 4) in vivo evidence for protective immunity related to the presence of serum antibodies to Polysaccharide II of Seibert (poly α(1->2) glucose. M. tuberculosis strains H37Rv, DT and Erdmann have been cultivated in synthetic media containing glycerol as the carbon source. The organism was inactivated with 70% ethanol and the surface structures removed by extraction in saline and then in Triton X-100. Both preparations showed almost pure polysaccharides containing glycogen and a cell wall saccharide, lipoarabinomannan. The cell pellet of strain Erdmann only released a saccharide reactive with pneumococcus type 12F antiserum. The precipitin reaction was similar to the reaction of this antiserum with dextran 1299 shown to have kojibiose, a di α(1->2) glucose. Several freshly isolated strains, from the blood and pleural fluid are being investigated for their content of the Polysaccharide II-like structure. Murine monoclonal antibodies, induced by immunization with inactivated M. tuberculosis and shown to have specificity for the α(1->2) glucose linkage are under investigation for their in vitro opsonphagocytic effect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00047-25 LDN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Studies of Neurons and Other Cell Types

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Brenneman	Head	LDN,NICHD
Others:	J. Hill	Expert	LDN, NICHD
	G. Glazner	IRTA Fellow	LDN, NICHD
	G. Gibney	IRTA Fellow	LDN, NICHD
	I. Gozes, R. Alvero	Guest Researchers	LDN, NICHD
	M. Koh, J. Wu	Guest Researchers	LDN, NICHD
	P. Gressens	Guest Researcher	LDN, NICHD
	D. Warren	Bio. Lab. Tech.	LDN, NICHD

COOPERATING UNITS *(if any)*

Biol. Psych. Br., NIMH (E. Sternberg); Dept. Organic Chem., Weizmann Inst. (M. Fridkin); Dept. Neurol., Univ. Kansas (B. Festoff); Dept. Biochem. George Washington Univ. (T. Moody);Dept. of Immunol. George Washington Univ. (T. Phillips); Tulane University (A. Arimura).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Developmental and Molecular Pharmacology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 7.6	PROFESSIONAL: 4.5	OTHER: 3.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activity Dependent Neurotrophic Factor (ADNF), a survival-promoting protein released from glial cells by vasoactive intestinal peptide (VIP), was found to be a 16 kDalton, monomeric protein with a pI of 8.2. ADNF has a unique amino acid sequence. The neurotrophic action of ADNF (EC50 < 1 fM) was isolated to a 15 amino acid peptide (ADNF-15). This peptide was synthesized and was found to be fully active in neuronal survival assays. Neutralizing antiserum was obtained to both intact ADNF (mouse polyclonal) and to ADNF-15 (rabbit polyclonal). Each antisera produced neuronal cell death in CNS cultures.

Administration of a VIP antagonist to pregnant mice (E9-E11) produced a severe microcephaly characterized by a 50% reduction in brain weight and DNA content. In animals with VIP antagonist-induced microcephaly, delays in developmental milestones were observed in 10 of 10 behavioral tests. Serum VIP levels in pregnant rats at mid-gestation showed a 6 to 10-fold increase in concentration, while alpha MSH and somatostatin remained unchanged.

A novel VIP agonist was synthesized and shown to be 100-fold more potent than VIP in producing neuroprotective actions in CNS cultures. This compound was shown to preferentially interact with VIP receptors in the CNS that mediate neuronal survival. VIP was shown to increase the release of eight cytokines in astrocyte cultures: M-CSF, G-CSF, IL-1 alpha and beta, IL-3, IL-6, TNF-alpha and interferon gamma. IL-1 alpha was found to have an age-dependent affect on neuronal survival in spinal cord cultures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00056-19 LDN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis, processing & secretion of neuropeptides & pituitary peptide hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Y. Peng Loh	Section Chief	LDN, NICHD
Others:	Le-Ping Pu	Visiting Fellow	LDN, NICHD
	Theodore Friedman	Med Staff Fellow	LDN, NICHD
	AnaMaria Bamberger	Visiting Fellow	LDN, NICHD
	David Cool	NRC Associate	LDN, NICHD
	Niamh Cawley	Pre-IRTA	LDN, NICHD
	Diane Louie	Pre-IRTA	LDN, NICHD
	Winnie Tam	Microbiologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Endocrinol. & Reprod. Res. Br. (H-C Chen); U of Auckland, New Zealand (N. Birch); Brown U (E. Nillni, I. Jackson); Sandoz Inst. Med Res. London (C. Snell)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.15	4.90	2.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The sorting signal motif that is both sufficient and necessary for targeting pro-opiomelanocortin (POMC, pro-ACTH/endorphin) to the regulated secretory pathway was identified. This motif consists of a 13 amino acid amphipathic heart-shaped loop with two lobes located at the N-terminus of POMC. The amphipathic loop contains four amino acids Asp-10, Leu-11, Glu-14, Leu-18 which are highly conserved across species and may be involved in the sorting mechanism by facilitating ionic and hydrophobic interactions with a putative sorting receptor. A novel class of aspartic protease capable of processing prohormones at paired and monobasic residue sites was studied. One of these is a yeast aspartic protease 3 (YAP3) which we have purified and characterized. This enzyme recognized three motifs in different prohormones studied. They are a pair of basic residues; and a pair or mono-basic residue, with an additional basic residue at the P4 to P6 position upstream or P4' to P6' position downstream from the cleavage site. The specificity and pH optimum of YAP3 were similar to the mammalian aspartic protease, pro-opiomelanocortin converting enzyme (PCE). PCE was shown to cross-react with a YAP3 antibody on Western blots suggesting that YAP3 and PCE are homologues. Immunocytochemistry using the YAP3 antibody showed immunopositive cells in neuropeptide-rich regions of mouse brain: the arcuate nucleus, paraventricular nucleus, and the pyramidal cells and granular cell layer in the hippocampus. YAP3-related cDNA clones have now been obtained from a mouse brain library and are currently being sequenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00064-18 LDN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobiologic Studies of Neurons and Glia in Cell Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.G. Nelson	Chief	LDN,NICHD
Others:	R.D. Fields	Snr Staff Fellow	LDN,NICHD
	R. Davenport	NRC Fellow	LDN,NICHD
	M.-X. Li	NRC Fellow	LDN,NICHD
	Y. Liu	Visiting Fellow	LDN,NICHD
	S. Fitzgerald	Biologist	LDN,NICHD
	A. Parfitt	Adjunct Scientist	LDN,NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.2	4.8	1.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have demonstrated activity-dependent synapse reduction in a 3-compartment tissue culture system in which two populations of cholinergic neurons converge to innervate a common population of muscle fibers. This synapse reduction is blocked by a specific thrombin inhibitor, hirudin; this implicates the action of thrombin in the process of synapse reduction. We now show that the source of the thrombin is the muscle or nerve cells (and not the serum usually present in the culture medium) by demonstrating activity-dependent synapse reduction in serum free medium. Exogenous thrombin produces synapse reduction even in the absence of neural activity and the thrombin induced reduction is blocked by hirudin. Thrombin takes some three days to produce synapse loss, while stimulation produces similar loss in only one day, so some synergy between thrombin action and nerve activity may occur. We have measured thrombin activity (hirudin sensitive proteolytic activity) in the supernatant media from inactive (tetrodotoxin silenced) and active (acetyl choline stimulated) muscle fibers. Activated muscle cells produce about twice as much thrombin as do inactive muscle. We have used semi-quantitative polymerase chain reaction methods to examine prothrombin mRNA levels in activated and inactive muscle cell cultures. After 48 hours of stimulation, prothrombin mRNA levels are about two times higher in activated than in inactive muscle cells. Thus in active muscle cells, and in correlation with the synapse loss that occurs with activation, thrombin secretion and the capacity for thrombin synthesis increases by some two fold. We have examined quantitatively the voltage-dependent Ca^{++} currents in dorsal root ganglion neurons, as a function of the activation of these cells. We find that the Ca^{++} currents are reduced substantially and significantly by 24 hours or more of electrical stimulation. This may be the basis for the adaptation of the growth cones of these cells to prolonged electrical stimulation that we had described earlier. mRNA for a cell adhesion molecule, L1, is strikingly down regulated by electrical stimulation of dorsal root ganglion neurons at only 0.1 Hz.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00094-24 LDN

PERIOD COVERED
October 1, 1993 - September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pineal Regulation: Environmental and Physiology Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	David C. Klein	Head	LDN, NICHD
Others:	Steven Coon	NRC Fellow	LDN, NICHD

COOPERATING UNITS *(if any)*
Georgetown University (M.A.A. Namboodiri); University of Geneva (N. Schaad); University of Giessen (H. Korf)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Neuroendocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The regulation of the pineal gland by environmental and physiological factors is analyzed, exclusive of transmembrane and intracellular regulatory mechanisms (see Z01 HD 00095-24 LDN). The pineal gland is part of the melatonin rhythm generating system, a neural circuit which includes a circadian clock in the suprachiasmatic nucleus (SCN); the SCN is reset and entrained by light acting through the eye. An area of special current interest is proteins involved in transmembrane signal processing. The most exciting finding is that there is a daily rhythm in the abundance of mRNA encoding the α-1B-adrenergic receptor. This increase seems to be driven by the SCN, and is blocked by light acting through the eyes. Studies in organ culture indicate the receptor is regulated by cAMP. This second messenger is controlled by several receptors, two of which have been found to mediate increases in the abundance of mRNA encoding the α-1B-adrenergic receptor. This work is important because it provides an interesting mechanism through which one receptor can regulate another. It establishes an interesting neural mechanism through which transmitter systems can interact.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00095-24 LDN

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pineal Regulation: Transsynaptic and Intracellular Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.C. Klein	Head	LDN, NICHD
Others:	J.L. Weller	Chemist	LDN, NICHD
	P. Roseboom	Staff Fellow	LDN, NICHD
	A. Lin	Visiting Fellow	LDN, NICHD
	R. Baler	Biotechnology Fellow	LDN, NICHD
	M. Bernard	Visiting Fellow	LDN, NICHD

COOPERATING UNITS *(if any)*

Georgetown Univ (M.A.A. Namboodiri); NEI (T. Shinohara); Univ of Giessen (H. Korf); NIMH (E. Ginns); LDN, NICHD (J. Russell, J. Hill)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neuroendocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.0	6.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mechanisms involved in the transduction of neural signals and the control of tissue specific gene expression are studied. The pineal and pituitary glands are used as model systems. The details of the chemical and ionic components of transmembrane signal processing and of neural and tissue specific regulation of gene expression are analyzed. Signal transduction in the pineal gland is of special interest because cAMP and cGMP are regulated by dual receptor mechanisms which appear to interact at the level of regulation of adenylyl and guanylyl cyclases. One leg of these pathways activates these enzymes via GTP binding regulatory proteins, similar to Gsα. In the area of the neural and developmental control of gene expression, advances have been made in purifying N-acetyltransferase and hydroxyindole-O-methyltransferase, and in isolating cDNA clones coding for these enzymes. It has been found that three HIOMT mRNA molecules exist, which appear to be the result of alternative splicing; one contains a truncated LINE sequence in the coding region. Recent studies have extended second messenger analysis into the role of transcription factors in signal transduction in the pineal gland.

The major hormonal product of the pineal gland is melatonin. Melatonin has been found to block GnRH induced increase in [Ca++]i and to block GnRH-induced depolarization. Melatonin appears to act on a subpopulation of GnRH-sensitive cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00704 -10 LDN

PERIOD COVERED

October 1, 1993 - September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tetanus Toxin Effects and Localization in Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Elaine A. Neale	Head	LDN, NICHD
Others:	Lura C. Williamson	Sr. Staff Fellow	LDN, NICHD
	Linda M. Bowers	Biologist	LDN, NICHD
	Sandra C. Fitzgerald	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)* Div. of Bacterial Products, Center for Biologics Evaluation & Research, Food and Drug Administration (J. H. Halpern and W.H. Habig); National Institute of Dental Research (S.H. Leppla); U.S. Army Medical Research Institute of Infectious Diseases (J.E. Brown); Dipartimento di Science Biomediche Sperimentali, University of Padova (C. Montecucco)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	1.4	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tetanus toxin is internalized into neurons by way of endosomes and is not trafficked through the Golgi apparatus, demonstrated by studies with monensin, bafilomycin, and brefeldin A. The low pH of the endosome appears necessary for translocation of tetanus toxin to the neuronal cytosol.

Immunoreactivity for VAMP/synaptobrevin, an integral membrane protein of synaptic vesicles thought to be the intracellular target of the catalytic domain of tetanus toxin, disappears from synaptic terminals in neuronal cultures treated with the toxin. Synaptic vesicles in toxin-blocked terminals dock but do not fuse with the presynaptic membrane.

Five of the seven serotypes of botulinum toxin block the release of glycine and glutamate in spinal cord cell cultures. The rank order of potency in the culture assay is similar to that in mouse injection assays. Competitive binding studies indicate that the E, but not the B, serotype interferes with the binding of serotype A, suggesting some homology in the binding domain of serotypes A and E.

A chimeric protein consisting of the transmembrane domain of anthrax toxin and the catalytic domain of tetanus toxin is cytotoxic (in the presence of the anthrax binding factor) to a number of non-neuronal cell lines, suggesting that the toxin acts to cleave other cellular proteins required for vesicle membrane fusion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00708-10 LDN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphologic Studies of Neuronal and Non-neuronal cells in CNS Cell Cultures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.A. Neale	Head	LDN, NICHD
Others:	A.G. Parfitt	Adjunct Scientist	LDN, NICHD
	L.M. Bowers	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Neurophysiology, NINDS, NIH (T.J. Smith, Jr.); Division of Bacterial Products, Center for Biologics Evaluation and Research, Food and Drug Administration (W.H. Habig and J.L. Halpern);

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.5	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Axonal, dendritic, or synaptic structures were identified by immunohistochemistry in developing neuronal cultures. In cultures where electrical activity was suppressed by chronic exposure to tetanus toxin or tetrodotoxin, synaptic structures were more abundant than in active cultures. Techniques are being developed for the intracellular injection of dyes into neurons in high density (maximally active) and toxin-exposed (electrically inactive) cultures in order to study dendrite development under these conditions during the first two weeks in culture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00709-07

PERIOD COVERED October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prevention of Neuronal Deficits Associated with AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Brenneman	Head	LDN,NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Developmental and Molecular Pharmacology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 HD 00710-06 LDN

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Characterization of Glutamate Receptor Expression in Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Buonanno	Head	LDN,NICHD
Others:	M. Sasner	IRTA Fellow	LDN,NICHD
	R. Beers	Biologist	LDN,NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Molecular Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.4	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activity-dependent neural plasticity during development and in the adult is largely mediated by N-methyl-D-aspartate receptor (NMDAR) activation; the natural ligand for this excitatory amino acid receptor is glutamate. During synaptogenesis, NMDARs have been found to play a major role in stabilizing neural connections in the visual system of mammals and lower vertebrates, and in registering somatosensory maps. In the adult, NMDA receptors have been implicated in the induction of long-term potentiation, a process characterized by the increase of synaptic efficacy in response to electrical stimulation. For these reasons, NMDARs provide an interesting target for understanding the maleable properties of the CNS. As a first step to elucidating the molecular mechanisms that target the expression of these family of receptors to specific neural populations during development, we have begun to analyze the expression of different receptor subunits during development and to dissect the transcriptional regulatory elements that modulate NMDAR gene expression. Using *in situ* hybridization histochemistry, we and others found that the εsubunits are differentially expressed during neurodevelopment. To begin elucidating the molecular mechanisms regulating NMDAR expression, the upstream region of the ε2 gene was cloned, sequenced and characterized. Multiple transcription initiation sites were mapped by RNase protection and a PCR technique based on primer extension. Transgenic mice harboring a chloramphenicol acetyltransferase (CAT) construct, driven by ε2 upstream sequences, expressed the reporter specifically in the brain. Experiments are in progress to delineate the *cis*-acting elements conferring developmental and neuron-specific transcription. The use of replication-deficient adenovirus vectors to introduce expression constructs into neurons has been investigated. This type of work provides the foundation for the future use of ε2 regulatory sequences in gene therapy experiments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00711-05 LDN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transcriptional Regulation of Skeletal Muscle-Specific Genes by Electrical Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Buonanno	Head	LDN, NICHD
Others:	S. Basu	IRTA	LDN, NICHD
	G. Gibney	NRC	LDN, NICHD
	J. Stauffer	Adjunct Scientist	LDN, NICHD
	R. Beers	Biologist	LDN, NICHD

COOPERATING UNITS *(if any)*

Dr. Eric Wourusek (NEI, NIH); L. Anglister (Hebrew Univ., Israel)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Molecular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20982

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.1	1.6	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Skeletal muscle plasticity is dramatically influenced by motoneuron innervation. We have identified muscle genes that are either repressed or stimulated by specific patterns of nerve-derived electrical activity; our goal is to elucidate the molecular mechanisms underlying activity-dependent transcriptional regulation. To this end, we previously demonstrated that myogenin, a muscle-specific factor that regulates transcription of nicotininc acetylcholine receptor (nAChR) genes in cultured cells, is repressed by innervation and de-repressed by denervation. Changes in myogenin levels in muscle preceded receptor regulation during development and after denervation, suggesting that regulation of myogenin may lie-upstream of receptor. Analyses of a 3.7 and 1.5 kb myogenin upstream region in transgenic mice have shown that these sequences confer muscle-, developmental-, and denervation-specific regulation. We have developed a modification of a myoblast implantation technique to map transcription regulatory sequences in mature skeletal muscle. Using this technique, we have delineated a 450 bp region that confers the denervation response.

To begin mapping the DNA sequences that stimulate the expression of the troponin I slow (TnI_s) gene in response to specific patterns of electrical activity, we have begun to map the promoter in cultured C2C12 muscle cells and transgenic mice. Delineation of TnI_s upstream sequences in cultured myocytes, demonstrated that 200 bp are necessary and sufficient to confer muscle- and developmental-specific transcription; MyoD and MEF-2 binding sites were shown to be required for activity. However, this region does not direct transcription of the gene in the adult slow muscles of transgenic mice; there is a requirement for sequences further upstream. We have begun to clone and characterize putative *trans*-acting factors that may regulate myogenin and TnI_s transcription. We have isolated cDNAs coding for a novel member of the *ets*-family and a rat homolog of MEF-2. The functional properties of these factors are currently under investigation. Analysis of the interactions of these families of *trans*-acting factors should help elucidate the molecular mechanisms underlying fiber-type specific regulation of muscle genes by innervation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00712-03 LDN

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of phenotypic differentiation in the developing mammalian CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. v. Agoston	Head	LDN,NICHD
Others:	A. Dobi	Visiting Fellow	LDN,NICHD
	E. Santha	Visiting Associate	LDN,NICHD
	R. Carbone	Adjunct Scientist	LDN,NICHD
	C. Palkovits	Lab Tech.	LDN,NICHD
	Ken Matsumoto	Visiting Fellow	LDN,NICHD

COOPERATING UNITS *(if any)*

LCB,NIMH (M. Palkovits); Dept. of Neurobiology, Duke University. (M. Riggott & W.D. Matthew); LNS, NINDS (A. Donevan & M. O'Donovan); LN, NINDS (B. Andrews), LNC, NINDS, (H. Chin); LN, NIDA (D. Vandenbergh); LBC, NHLBI (J. Silverton).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Molecular Control of Neurodifferentiation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular mechanism of phenotypic establishment was investigated by studying embryonic specific protein-DNA interactions and structural changes of the DNA during neuronal differentiation using the enkephalin (ENK) gene as model gene. DNA methylation interference footprinting was used to identify a novel (TTTGCAT) octamer-like motif (sept) and its binding protein which is dissimilar to oct-1 or oct-2, phosphorylated, brain and neuron-specific and showed strong correlation with the ontogeny of brain regions. The effect of Ca2+ on the DNA structure containing the di-nucleotide repeat (TG/AC)28 was further investigated by osmium tetroxide (OsO4) footprinting and supported earlier findings that Ca2+ could induce a concentration-dependent change in DNA structure mediated by (TG/AC)n motif which are abundant in the genome. Using rapid freeze fixation of embryonic neurons followed by electron probe X-ray microanalysis the intranuclear physiological Ca2+ concentration was determined at around 1 mM. Two brain- and development-specific (TG/AC)n repeat binding proteins were identified, one present only in E14 striatal primordium, the other is present in all regions of the embryonic brain between embryonic ages E14 and E18. A novel magnetic bead based assay system was developed for studying protein based far-distant DNA-DNA interactions. A far-upstream region (-2000 to -2800 bp relative to the start site of the rat ENK gene) showed development- and brain region--specific, protein based interaction with the core promoter/TATA region. Within this region a novel trinucleotide repeat (ATT)19 located around -2450 bp of rat ENK gene was identified by DNAse I footprinting interacting with the proximal promoter/TATA region. Mobility shift assay demonstrated that the (ATT)19 repeat binding protein is brain-, neuron-, development- and brain region specific and furthermore negatively correlates with ENK expression. An RT PCR based assay was established to follow the developmental changes in the expression pattern of various markers in different regions of the rat brain. A primary developing neuronal culture system in conjunction with lipofectin-based DNA transfer was established to study cis-requirements of the ENK gene and to load neurons with double stranded decoy DNA to investigate the role of identified motifs in ENK expression. Developmental changes in the methylation of the rat ENK gene was studied by PCR and a development- and tissue-specific changes in DNA methylation was established. Genomic PCR analysis of various mouse and human phenotypes was performed and showed unusually low extent of polymorphism of the (TG/AC)n repeat of both mouse and human ENK gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01202-7 LDN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of expression and function of neuropeptides during development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Y. Peng Loh	Section Chief	LDN, NICHD
Others:	William P. Hayes	IRTA	LDN, NICHD

COOPERATING UNITS *(if any)*

Lab. Biol. Chem., NCI (J. Battey), Lab. Neurochem., NINDS (S. Wray, J. Mill and H. Chin); Univ of Montreal (J. Chan); Lab. Cell. Mol. Neurophys., NICHD (V. Gallo)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the past year, the project on the developmental expression of neuropeptide genes in the Xenopus CNS was discontinued. A study on the developmental expression of one member of the LIM class family of homeobox genes was carried out in rat cerebellum, in collaboration with Dr. V. Gallo in LCMN. Northern blot analysis showed that rat rLIM-1 expression in cerebellum was at its peak level at postnatal day 8, coincident with a peak period of granule cell proliferation. A dramatic decrease was observed in rLIM-1 expression by postnatal day 30. In situ hybridization histochemistry showed that in adult rats, Purkinje cells express high levels of rLIM-1 but internal granule cells express low levels. In developing rat cerebellum, proliferative and premigratory cells express high levels of rLIM-1 but down regulates in post-mitotic cells, coincidental with onset of migration through the molecular layer. These findings suggest that rLIM-1 may play a role in inducing neurona progenitor cells and their progenies to adopt the granule cell fate in developing rat cerebellum. Project#Z01HD01202-6 LDN is now completely terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01900-04 LME

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Regulation of Differential Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Alan Wolffe, Visiting Scientist LME/NICHD
OTHERS
Philippe Bouvet, Special Volunteer LME/NICHD
Mary Dasso, Staff Fellow LME/NICHD
Stefan Dimitrov, Visiting Scientist LME/NICHD
Lita Freeman, Special Volunteer LME/NICHD
James Godde, IRTA Fellow LME/NICHD
Jeffrey Hayes, IRTA Fellow LME/NICHD
Stefan Kass, Special Volunteer LME/NICHD
Nicoletta Landsberger, Special Volunteer LME/NICHD
Funda Meric, Special Volunteer LME/NICHD
Karl Nightingale, Visiting Fellow LME/NICHD
Danielle Patterton, Visiting Associate LME/NICHD
Dmitry Pruss, Visiting Fellow LME/NICHD
Monika Puzianowska-Kuznicki, Visiting Fellow LME/NICHD
Hisato Saitoh, Visiting Fellow LME/NICHD
Yun-Bo Shi, Senior Staff Fellow LME/NICHD
Melissa Stolow, Special Volunteer LME/NICHD
Donald Stump, IRTA Fellow LME/NICHD
Kiyoe Ura, Special Volunteer LME/NICHD
Jiemin Wong, Visiting Fellow LME/NICHD

COOPERATING UNITS *(if any)*

Dept. Microbiology, SUNY (W.R. Bauer); Dept. Mathematics, UCLA (J. White); Lab. of Mol. Carcinogenesis, NCI (M. Bustin); Dept. Biochem. Southwestern Med Cener (J. Hansen); Lab. of Chem. Physics (M. Clore, A.M. Gronenborn)

LAB/BRANCH

Laboratory of Molecular Embryology

SECTION

Section on Molecular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD. 20892-2710

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
18	18	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our work is focussed on the molecular mechanisms responsible for establishing and maintaining stable states of gene expression during vertebrate embryogenesis.

Progress has been achieved in the following key areas:

1. We have found that histones can exert dominant repressive effects on the transcription of a gene in vivo in spite of an abundance of transcription factors for that gene.

2. We have determined that the temporal regulation of transcription during early embryogenesis will depend upon the capacity of a specific transcription factor to bind to DNA in the presence of core histones and to recruit TBP to a promoter in a competition for binding with the core histones.

3. We have found that the deformation of DNA by the core histones increases its utilization by the human immunodeficiency virus (HIV) integrase.

4. We have discovered that the translation process is functionally coupled to transcription in eukaryotes.

5. We have demonstrated that the nuclear proteins RCC1 and Ran are directly involved in signal transduction pathways preventing the initiation of premature mitosis.

6. We have characterized thyroid hormone response elements in the gene encoding the thyroid hormone receptor.

7. We have found that the morphogen hedgehog is reputated by thyroid hormone during metamorphosis.

0

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00066-24 LMG

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control Mechanisms in Temperate Bacteriophage λ

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert A. Weisberg	Section Head	LMG:NICHD
Others:	Banik-Maiti, Sarbani	Visiting Fellow	LMG:NICHD
	Dorgai, Laszlo	Visiting Associate	LMG:NICHD
	King, Rodney	NRC Fellow	LMG:NICHD
	Sloan, Sieghild	Microbiologist	LMG:NICHD
	Yagil, Ezra	Prof. Serv. Contract	LMG:NICHD

COOPERATING UNITS *(if any)*

Department of Biochemistry, Tel Aviv University, Israel (Ezra Yagil); Laboratory of Molecular Biology, NCI (Ding Jun Jin); Division of Biomedical Science, Brown University (Arthur Landy).

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.2	4.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The integrase proteins of phages λ and HK022 are closely related site-specific recombinases that recognize different nucleotide sequences in the core regions of their substrates, the attachment sites of the two phages. The two proteins differ by 92 amino acid substitutions. We have found that no more than 4 of these substitutions are critical for the different DNA sequence specificities of these enzymes. Two of these substitutions act principally by reducing specificity, while another acts principally by specifically decreasing activity on one of the substrates. Using a variant λ integrase with HK022 core binding specificity, we have tested the hypothesis that cleavage of the core region during recombination results from nucleophilic attack on the DNA by an amino acid residue contributed by an integrase protomer bound to a nearby site ("trans cleavage"). Our results show that an integrase protomer bound to the site that is cleaved can contribute the residue used in nucleophilic attack ("cis cleavage"). This result argues that the integrase catalytic site is not shared between protomers. We have continued our studies on the mechanism of transcription antitermination in bacteriophage HK022. The only requirements known for antitermination in this system are cis-acting phage sequences and the integrity of a putative zinc binding region in the β' subunit of *E. coli* RNA polymerase. We have developed a genetic selection for isolating and characterizing mutants that affect antitermination. Changes affecting the first 80 nt of the phage pL transcript as well as changes within the nontranscribed region of the promoter reduce pL operon antitermination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00067-26 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Integration of Macromolecular Synthesis in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Cashel, MD PhD	Section Chief	LMG:NICHD
Others:	D. Gentry, PhD	IRTA	LMG:NICHD
	V.J. Hernandez, PhD	IRTA	LMG:NICHD
	H. Murphy	Chemist, Lab. Tech.	LMG:NICHD

COOPERATING UNITS *(if any)*

Dr. R.R. Burgess, McArdle Lab. for Cancer Research, Madison, WI; Dr. Gad Glaser, Hadassah Med. School, Jerusalem, Israel; Dr. Horst Malke, Inst. for Mol. Biol., Jena Univ, Jena, Germany; Dr. Arthur Kornberg, Stanford Univ. Med. Ctr., Stanford

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Molecular Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.9	3	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves understanding how changes in Escherichia coli gene expression are coordinated when cell growth is limited by nutrient availability. We are interested in signals provoking changes in cellular levels of a class of regulatory nucleotide analogs of GDP and GTP that bear pyrophosphate residues on the ribose 3' hydroxyl; abbreviated as ppGpp and pppGpp, respectively, or (p)ppGpp collectively. Changes in (p)ppGpp levels are of interest because they are correlated with complex changes in gene expression triggered by sensing starvation for sources of energy, of amino acids or of phosphate. Since (p)ppGpp is not essential for growth, it can be argued that these nucleotides provide predominately regulatory functions for the cell. We reported that (p)ppGpp induces expression of the alternative RNA polymerase sigma factor, sigma-S, encoded by the rpoS gene. This is significant because sigma-S accumulation triggers entry into a globally altered pattern of gene expression characteristic of stationary phase. We now find that (p)ppGpp induction of rpoS occurs at the translational level, a distinctly new level of regulation for (p)ppGpp; (p)ppGpp typically affects transcription. (p)ppGpp-independent mutants of the "housekeeping" sigma factor, encoded by rpoD, have been shown to affect patterns of abortive RNA chain release during in vitro transcription initiation, implicating a promoter clearance mechanism. We continue to explore regulatory relations described between (p)ppGpp, phosphate availability, and polyphosphate (Pn). The functional and sequence relatedness between the pppGpp gamma phosphohydrolase, encoded by gppA, and the major Pn phosphatase, encoded by ppx, has been extended to include preliminary evidence of regulatory parallels as well. Phosphate limitation provokes well known global changes in gene expression owing to activation of a DNA binding protein (PhoB) by phosphorylation as well as (p)ppGpp induction; leading to induction of rpoS. We now find that mutants eliminating either (p)ppGpp or eliminating rpoS alter Pho regulon activity. We have indications that a stationary phase-specific Pn phosphatase may exist in addition to the ppx encoded enzyme expressed during growth. The operon containing ppk and ppx so far known to be involved in Pn synthesis and degradation respectively, has been characterized by the Kornberg laboratory. We have constructed ppkx deletions and shown them to be lethal when combined with a deletion of rpoS. We suspect the rpoS governs stationary phase sources of Pn synthesis and ppkx governs sources of Pn synthesis during exponential growth; Pn may be an essential cell component. To test this, we are collaborating with the Kornberg laboratory, experts in biochemical characterization of Pn synthesis and degradation activities. We are characterizing putative ATP-dependent RNA helicase genes; at least five such "DEAD box" genes exist in E. coli and multiple genes are found in all organisms tested. Deletion analysis gives us the first indication that such genes may provide essential functions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00068-23 LMG

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Factors Influencing Genetics Transcription-Initiation and Termination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert J. Crouch, Ph.D.	Senior Investigator	LMG:NICHD
Others:	Don G. Ennis	Senior Staff Fellow	LMG:NICHD
	Susana Cerritelli	Senior Staff Fellow	LMG:NICHD
	Wu-Po Ma	Visiting Fellow	LMG:NICHD
	Yuzuru Tozawa	Fogarty Fellow	LMG:NICHD
	Zhan, Xinyi	Fogarty Fellow	LMG:NICHD

COOPERATING UNITS *(if any)*

Section on Viral Gene Reg., LMG:NICHD; Protein Expression Lab., OD:NICHD; Dr. Valarie Mizrahi, Mol. Biol Unit, The S. African Inst. for Med. Res., Johannesburg; Dr. Marc Drolet, Dept. of Microbiol & Immunol, Univ of Montreal, Canada

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Formation of RNA

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.75	5.75	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ribonuclease H (RNase H) plays crucial roles in replication of retroviruses, including the human immunodeficiency virus (HIV) as well as involvement in normal cellular events including DNA replication. The purpose of this project is to gain a detailed understanding of the precise nature of the substrates for RNases H, the chemical makeup of cellular RNases H and the effect of either producing more or less RNase H (or a more or less active RNase H) on viral and cellular DNA synthesis. We have found that two eukaryotic enzymes have, in addition to RNase H, a double-stranded RNA (dsRNA) binding domain that is not present on retroviral or bacterial RNases H. Unlike other dsRNA-binding motifs, a single copy of the dsRNA-binding domain is necessary and sufficient for binding to dsRNA. For one of these RNases H, we have found that each domain can function independently but when on the same polypeptide each domain has rather dramatic influences on the properties of the other domain. This unexpected association of RNases H with a dsRNA-binding region suggests a possible coupling between DNA and protein synthesis (the latter of which is known to be affected by dsRNA).

We have expressed bacterial RNase H in cells in such a manner that it gets incorporated into retrovirus-like particles in yeast. The purpose of this set of experiments is to better understand how the two types of RNases H (retroviral and bacterial) differ and secondly, to see at what stage the more potent bacterial enzyme might inhibit propagation of the virus-like particle. We have observed a rather strong inhibition of the ability of the retroviral DNA to move from one site to another (transposition). Having the stronger acting bacterial RNase H in the viral particle appears to interfere with replication of the viral DNA within the particle.

DNA topology is also a particularly important problem related to RNase H. During the course of transcription, RNA-DNA hybrids can form and displace one of the DNA strands forming R-loops. Such R-loops, in theory can also create topological problems for the DNA. We have shown that there is some interaction between DNA topoisomerase I, DNA gyrase and RNase H in bacterial cells to control the amount of topological "super-coiling" of DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00069-22 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Mammalian Retrovirus Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.G. Levin	Research Biochemist	LMG:NICHD
Others:	J. Guo	IRTA Fellow	LMG:NICHD
	M. Powell	IRTA FELLOW	LMG:NICHD
	W. Wu	Visiting Fellow	LMG:NICHD
	Z.Y. Yuan	Guest Worker	LMG:NICHD
	Y.-X. Feng	Guest Worker	LMG:NICHD
	K. Post	Biologist	LMG:NICHD

COOPERATING UNITS *(if any)*

NICHD-LMG (R. Crouch); ABL Basic Research Program, NCI-FCRDC (A. Rein); CBER-DV (C. Marcus-Sekura)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Viral Gene Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.9	3.9	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to define the molecular mechanisms involved in the replication of mammalian retroviruses and in particular, to understand the factors which influence the regulated expression of viral genetic information. Studies are being carried out on the functional relationship between the polymerase and RNase H domains of reverse transcriptase (RT). We have been investigating the effect of replacing the viral RNase H domain with a cellular RNase H on processive DNA synthesis, using templates having sequences from the polypurine tract, an essential region at the 3'-end of the viral genome. Two chimeric RTs with the entire murine leukemia virus (MuLV) polymerase domain or all but the last 19 amino acids, respectively, fused to *E. coli* RNase H have been expressed and purified. No major differences in the activities of the two chimeric RTs are detected, indicating that the additional 19 amino acids, which form the C-terminus of the connection subdomain, do not significantly influence the enzyme function of these mutants. The chimeric RTs as well as an RT mutant missing the entire RNase H domain are deficient in catalyzing processive DNA synthesis, although the effect is more pronounced with the RNase H-minus mutant. The results of band shift assays indicate that this defect reflects inefficient binding of the mutant RTs to primer-template. Moreover, unlike wild-type RT, which appears to be active in a dimeric form during DNA synthesis, mutant reactions contain mostly RT monomers. We conclude from these findings that (i) the presence of a wild-type polymerase domain is not sufficient by itself to ensure processive DNA synthesis; (ii) the viral RNase H domain stabilizes RT binding to primer-template, which in turn facilitates formation of the active form of RT and processive DNA synthesis; and (iii) an RT with a heterologous RNase H is more processive than an RT without any RNase H domain. Other studies on MuLV RT involve genetic and biochemical approaches to explore the influence of the connection subdomain on polymerase and RNase H activities and to investigate factors which affect enzyme pausing during primer extension. Studies on HIV-1 RT are focusing on the requirements for site-specific cleavage at the 3'-end of the polypurine tract and initiation of plus-strand viral DNA synthesis. Sequence requirements for in vitro dimerization of viral genomic RNA and the role of nucleocapsid protein in this process are also being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01002-12 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression During Embryonic Development of Xenopus Laevis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I.B. Dawid	Head (All Personnel Listed LMG:NICHD)		
Others:	A. Karavanov	Vis. Scientist	M. Rebagliati	IRTA Fellow
	M. Taira	Vis. Scientist	M. O'Connell	IRTA Fellow
	R. Toyama	Vis. Associate	M. Rebbert	Chemist
	J-P. Saint-Jeannet	Vis. Fellow	P. Curtiss	Biologist
	E. Glasgow	NRC Fellow	W. Hane	Guest Researcher
	P. Good	IRTA Fellow	N. Sugimoto	Volunteer
	C. Jungwirth	Courtesy		

COOPERATING UNITS *(if any)*

K. Geering and B. Rossier, Lausanne; W. Hayes, LDN, NICHD; H. Westphal, LMGD, NICHD; R. Harland, U.C. Berkeley; K. Ozato, LMGR, NICHD; K. Cho, UC Irvine; Xi He & H. Varmus, NIH

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
11.7	9.6	2.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular mechanisms in embryogenesis of the amphibian *Xenopus laevis* and the zebrafish have been studied. Pattern formation is controlled in the embryo by the action of cell signalling molecules that elicit the expression of transcription factor-encoding genes in a spatially and temporally regulated way. The focus of this laboratory has been the study of the LIM class of homeobox genes whose members are instrumental in pattern formation and tissue differentiation. Three LIM genes have been analyzed in detail.

The *Xenopus Xlim-1* gene and the homologous zebrafish and mouse *lim1* genes, are expressed in three distinct lineages: (1) The dorsal mesoderm during gastrulation, an area know as Spemann's organizer in the frog. Expression in this area is responsive to the mesoderm inducer activin. (2) The pronephros. (3) Specific regions of the CNS. Expression in regions 2 and 3 is sensitive to retinoic acid (RA). The function of *Xlim-1* has been sudied by ectopic expression in *Xenopus*. The results indicate that (i) the cysteine-rich LIM domains are negative regulatory domains, so that wild type Xlim-1 protein is largely inactive; mutations in the LIM domains reveal its functional potential. (ii) Mutated Xlim-1 can activate neural markers in naive cells, and muscle markers in cooperation with Xbra. Both of these activities are mediated through intercellular communication. (iii) Mutated Xlim-1 activates, probably directly, the *goosecoid* gene, another homeobox gene important in early embryogenesis. Activin responsive transcriptional regulation of the *Xlim-1* gene appears to be based on a constitutive promoter and an activin-sensitive silencer located in the first intron.

The *Xlim-2* and zebrafish *lim2* genes are very similar to *Xlim-1/lim1*, but the expression pattern is very different. *Xlim-2/lim2* is expressed in the entire ectoderm at early stages, and subsequently becomes restricted to the diencephalon and certain cells in the spinal cord. *Xlim-2* expression is not responsive to activin or RA. The pattern of the *Xlim-3* gene has been described in the previous Report. Zebrafish and mouse *lim3* genes have similar expression patterns; the early and high-level expression in the pituitary is of particular interest.

Mechanisms of cell-to-cell signalling in early embryogenesis has been studied in frogs and zebrafish. Evidence has been obtained for the involvement of glycogen synthase kinase-3 (GSK-3) the dorsalizing signalling cascade in *Xenopus*. In the zebrafish embryo, overexpression of the nodal protein, a growth factor in the TGF-β family, leads to duplication of the dorsal axis. This duplication is initiated by the formation of an ectopic dorsal center as visualized by the expression of the *goosecoid* and *lim1* genes. A role for a nodal-like factor in fish embryogenesis is suggested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01004-11 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Amino Acid and Nucleotide Biosynthesis in Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A.G. Hinnebusch, Research Microbiologist, (All listed personnel LMG:NICHD)

C. Grant	Vis. Fellow	M. Marton	NRC Fellow	G. Pavitt	Guest Researcher
C. Vasquez	Vis. Fellow	P. Romano	IRTA Fellow	M. Garcia	" "
W. Yang	Vis. Assoc.	R. Rolfes	IRTA Fellow	E. Duenas	" "
T. Dever	NRC Fellow	B. Jackson	Biologist	K. Natarajan	Vis. Fellow
C. Drysdale	NRC Fellow	M. Pak	NRC Fellow		

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Molecular Genetics of Lower Eukaryotes

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
140.20	13.20	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

GCN4 is a transcriptional activator of amino acid biosynthetic genes in S. cerevisiae that is repressed at the translational level by four short upstream open-reading-frames (uORFs). In cells starved for an amino acid or purine, ribosomes translate uORF1, bypass the start codons at uORFs 2-4, and reinitiate translation at GCN4 instead, because they fail to rebind a ternary complex of initiation factor-2 (eIF-2), GTP and charged initiator tRNA^{Met} until after scanning past uORF4. The concentration of ternary complexes is reduced in starved cells by phosphorylation of the α subunit of eIF-2 by GCN2. Several lines of evidence indicate that phosphorylated eIF-2 regulates translation in yeast by inhibiting guanine nucleotide exchange on eIF-2 by eIF-2B. Point mutations in the GCN3, GCD7, and GCD2 subunits of eIF-2B uncouple GCN4 translation from eIF-2α phosphorylation. These mutations cluster in a region of homology between the three protein that may define the sites of interaction between eIF-2B and eIF-2(αP). Overexpression of only these three eIF-2B subunits reverses the effects of eIF-2α hyperphosphorylation, suggesting that they form a subcomplex of eIF-2B components that can sequester eIF-2(αP). The effects of eIF-2α hyperphosphorylation is also suppressed by overexpressing the three subunits of eIF-2, even though this leads to higher absolute levels of eIF-2(αP), the inhibitor of eIF-2B. This establishes that eIF-2(αP) inhibits eIF-2B by competitive inhibition rather than by non-irreversible binding of eIF-2(αP) to eIF-2B. GCN4 translation can be induced by decreasing the dosage of initiator tRNA^{Met} genes, providing in vivo evidence that phosphorylation of eIF-2 reduces the level of ternary complexes. In addition to a requirement for A+U-rich sequences surrounding the uORF1 stop codon, translational reinitiation at GCN4 is dependent on sequences in the leader preceding uORF1. The GCN1 and GCN20 proteins are required in vivo for phosphorylation of eIF-2α by GCN2, and GCN1 and GCN20 were found to be components of the same high molecular weight complex. The requirements for dsRNA binding in vitro and translational control in yeast cells by the human dsRNA-activated eIF-2α kinase (DAI) strongly coincide, providing in vivo evidence that the level of DAI activation is largely a function of its ability to bind dsRNA. The identification of dominant-negative alleles and interallelic complementation involving different DAI mutants indicates that the active form of DAI in yeast cells is a homodimer. The transcriptional activation domain of GCN4 contains two large subdomains, each of which is sufficient for nearly wild-type transcriptional activation in vivo and is dependent on the co-activator ADA2. Each subdomain contains several smaller units, consisting of bulky hydrophobic residues surrounded by acidic amino acids, that cooperate in different combinations to activate transcription. Adenine-repressible transcriptional activation in yeast has been conferred on the CYC1 promoter with an ca. 80 bp fragment from the ADE5,7 gene containing two consensus BAS1 binding sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01005-07 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cellular Proliferation and Diversity in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Kennison, Ph.D.	Senior Staff Fellow	LMG:NICHD
Others:	A. Felsenfeld, Ph.D.	Senior Staff Fellow	LMG:NICHD
	M. Vásquez, Ph.D.	Visiting Fellow	LMG:NICHD
	B. Brizuela	Bio. Lab. Tech	LMG:NICHD
	T. Brown	SIS	LMG:NICHD

COOPERATING UNITS *(if any)*

Dr. J. Tamkun, Department of Biology, University of California, Santa Cruz
Dr. D.-H. Huang, Institute of Molecular Biology, Academia Sinica, Taiwan

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.4	1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genes involved in the determination and differentiation of adult tissues in *Drosophila melanogaster* have been identified by genetic interactions with homeotic and segmentation genes already known to be required in these tissues. Many new genes required for transcriptional activation of developmental genes have been identified. One of these new genes, *brahma*, encodes a large nuclear protein conserved from yeast to man. *brahma* mutations have in turn been used to identify other interacting genes required for transcriptional activation. The *osa* gene, which shows allele-specific interactions with *brahma* mutations, has been cloned by transposon tagging, and encodes a single 3 kb transcript. We have shown that maternal expression of both *brahma* and *osa* is essential for early embryogenesis, while zygotic expression of both is essential late in embryogenesis and during larval growth. Both genes are expressed primarily in the nervous system at later embryonic stages. This is also the tissue that expresses the highest levels of the homeotic genes that *brahma* and *osa* regulate. *brahma* and *osa* function are also required in the larval tissues for expression of the *hedgehog* gene, which encodes a secreted protein involved in cell-cell signaling during development.

The RNA polymerase II transcription factor TFIID is a complex of at least nine polypeptides. We have isolated mutations in the *Taf60* gene that encodes the 60 kDa subunit of this protein complex. The *Taf60* mutations have allowed us to determine several interesting features about the function of this protein complex in development. Maternal expression of the gene is required for oocyte formation. This maternal expression appears to be sufficient for normal embryogenesis. Although zygotic expression is essential for viability, it is not required until the larval stages. Our dosage studies with the *Taf60* mutations also suggest that while the TFIID complex may be required for transcriptional activation, its availability is probably not a limiting factor for transcriptional regulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01006-06

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein-Nucleic Acid Interactions in Vertebrate Embryogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	T. Sargent	Research Microbiologist	LMG:NICHD
Others:	K. Deonauth	Pre IRTA Graduate Student	LMG:NICHD
	J. Franklin	IRTA Fellow	LMG:NICHD
	M. Morasso	IRTA Fellow	LMG:NICHD
	R. Winning	Visiting Fellow	LMG:NICHD
	J. Scales	NRC Biotechnology Fellow	LMG:NICHD

COOPERATING UNITS *(if any)*

K. Mahon, LMGD, NICHD

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Vertebrate Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general aim of this project is to identify, characterize and study the function of protein factors that regulate vertebrate embryonic development. This is being approached in three ways. One approach is to analyze the expression and regulation of a vertebrate homolog of the *Drosophila* gene *Distal-less*. In flies this gene regulates limb and sensory appendage formation. The frog and mouse homologs are expressed in gradients in the limb epithelia, and also in hair follicles and tooth primordia. DNA elements and protein-DNA interactions responsible for this expression pattern are being mapped. This information will be used to test for the function of *Distal-less* gene expression in frog and transgenic mouse embryos.

A second approach employed has been to clone mRNAs encoding protein kinases that are expressed in the early frog embryo. Two such proteins of the receptor tyrosine kinase class, and one cytoplasmic serine-threonine kinase have been isolated and are being studied in frog embryos and in cultured mammalian cells.

The third approach focuses on the developmental function of calcium-dependent cell adhesion molecules in the zebrafish neural tube. A novel cadherin, VN-cad, has been cloned, sequenced, and characterized by in situ hybridization. It is being used in "dominant negative" RNA injection experiments to determine its function in zebrafish neurogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01008-05 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Protein-Nucleic Acid Interactions in Drosophilia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S.R. Haynes	Expert	LMG:NICHD
Others:	V.M. Heatwole	IRTA Fellow	LMG:NICHD
	D.T. Stolow	NRC Research Associate	LMG:NICHD
	M.T. Cooper	Biologist	LMG:NICHD

COOPERATING UNITS *(if any)*

Ann Beyer, Department of Microbiology, University of Virginia Medical School

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Protein-Nucleic Acid Interactions

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	3.0	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to understand the functions of *Drosophila* RNA binding proteins, particularly those related to the vertebrate A/B group hnRNP proteins, a major class of nuclear proteins that bind pre-mRNA. Biochemical studies of vertebrate hnRNP proteins suggest that they are involved in packaging and splicing nascent transcripts. We use genetics and molecular biology to study six *Drosophila* proteins that have similar RNA binding domains, two of which, *Hrb98DE* and *Hrb87F*, are hnRNP proteins. Four of these proteins are required for male fertility.

We isolated a small deletion that removes the *Hrb87F* gene and part of an adjacent gene, *tsr*. The only obvious phenotype associated with this deletion is that males are semisterile. The *tsr* protein has RNA binding motifs, and can bind RNA in vitro. The *tsr* gene is transcribed only in the male germ line, beginning during the third larval instar. Experiments using fly strains containing transgenic copies of wild type *Hrb87F* or *tsr* genes have shown that the disruption of the *tsr* gene is responsible for the sterile phenotype. In an otherwise wild type background, *Hrb87F* is not required. However, genetic interaction experiments show that it does affect male fertility under conditions of reduced dosage of *Hrb98DE*.

Mutations in the *Rb97D* gene, another RNA binding protein, also lead to male sterility. These mutations affect the expression of a dynein protein, an important component of sperm tails. The phenotype of the *Rb97D* mutants is very similar to that of a deletion of the dynein gene on the Y chromosome, suggesting that we have identified a target of the *Rb97D* protein.

The *cabeza* gene encodes an RNA binding protein that is a *Drosophila* homolog of several human proteins that are disrupted in translocations associated with some sarcomas. Several transcripts are produced from the *cabeza* locus, including one that appears to lack the RNA binding domain. The *cabeza* protein is predominantly nuclear, enriched in the embryonic brain and nerve cord and in the adult head, and is expressed in the nerve cord and imaginal discs in larvae. The *cabeza* gene has been mapped to 14B, and a genetic analysis of this locus is underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01009-02 LMG

PERIOD COVERED

October 1, 1994 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation and Function of Genetic Elements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H.L. Levin	Senior Staff Fellow	LMG:NICHD
	M.J. Benedik	Sabbatical Visitor (IPA)	LMG:NICHD
	J. Lin	IRTA	LMG:NICHD
	A. Atwood	Bio Lab Tech	LMG:NICHD

COOPERATING UNITS *(if any)*

Dr. Jef Boeke, Johns Hopkins University, Baltimore, Maryland
Dr. Sandra Martin, University of Colorado Health Sciences Center, Denver
Dr. Michael Benedik, University of Houston, TX

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Yeast Molecular Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.65	2.65	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our research program focuses on the mechanisms of retroelement action. Our approach to understanding the complex interactions between the retroelement and its host is to study retrotransposons, a family of elements that are closely related to retroviruses. A significant advantage to studying retrotransposons is they exist in hosts such as yeast that can readily be studied using sophisticated molecular genetic techniques.

Using our improved *in vivo* transposition assay, we have collected strong evidence that Tf1 reverse transcriptase uses a novel self-priming mechanism to initiate cDNA synthesis. This is in complete contrast to the tRNA mechanisms thought to be used by all other LTR-containing elements. Analysis of other transposons indicated that the self-priming mechanism of Tf1 defines a new family of retroelements.

The assembly of functional Tf1 particles has been a paradox since other retroelement particles assemble with a molar excess of capsid protein that accumulates because the levels of the Pol proteins are restricted by reading frameshifts or stop codons. Tf1 however, expresses all its protein from within a single open reading frame as a primary translation product. Because there is no obvious mechanism for overproducing Gag protein, we wondered if Tf1 particles contained equal amounts of Gag and Pol proteins or if there is a specific postranslational process that adjusts the ratios to allow for assembly. Careful quantitative measurements made this year indicate Tf1 particles contain a geater than 20-fold excess of Gag over IN. By looking at cultures in different stages of growth, we have been able to observe a degradation process that leads to this excess of Gag.

To determine the factors required for transposition, we have created two large sets of mutant strains that are defective for transposition. One collection of mutants was generated by treating the host cells with a mutagen while the other set was made by creating mutations in Tf1 itself. We are now in the process of analyzing both sets and hope as a result to identify host genes that are intimately involved in transposition. Thus far, we have identified two host mutants that are defective for the degradation mechanism required to create the molar excess of Gag to Pol proteins.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD-00071-22-LMGD

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene and Transgene Regulation in the Developing Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H. Westphal, M.D., Chief, LMGD, NICHD
A. Agulnick, Ph.D., IRTA
S. Bertuzzi, Ph.D., VF
J. Drago, M.D., Ph.D., GW
T. Fujii, M.D., Ph.D., VF
A. Grinberg, D.V.M., Veterinarian
B. Mosinger, Jr., M.D., Ph.D., VA
T. Nakamura, M.D., Ph.D., VA
J. Pichel, Ph.D., VF
F.D. Porter, M.D. Ph.D., SSF
H. Z. Sheng, M.D.,Ph.D., VA
A. Zhadanov, Ph.D., VF

COOPERATING UNITS *(if any)*

SDB, LMG, NICHD, NIH; GB, NIDDK, NIH; LCB, NIMH, NIH; HHMI, Children's Hospital, Boston, MA

LAB/BRANCH
Laboratory of Mammalian Genes and Development

SECTION
Section on Transgene Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
15	11	4

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Murine LIM-homeobox genes involved in the development of central nervous and neuroendocrine tissues. This gene family encodes regulators of development that play pivotal roles in pattern formation and cell specification during metazoan development. Their products contain two N-terminal cysteine-rich motifs, the LIM domains, and an adjacent homeodomain. This group has cloned and sequenced murine homologs of the Xenopus laevis genes XLIM1, XLIM2 and XLIM3. The degree of homology is very high. LIM1 expression in the developing mouse embryo begins during gastrulation and later homes in on the central nervous system from the telencephalon through the spinal cord, and on the developing excretory system. The LIM3 gene is expressed in the developing pituitary gland, in the ventral hindbrain and the ventral spinal cord. The pattern of LIM2 expression is currently under investigation. Also, deletions in two murine lim genes have been generated in the mouse germline in an effort to study their mechanism of action during mouse development.

Effects of human p53 expression on murine lens development. The wild-type human p53 gene causes microphthalmia when targeted to the developing embryonic lens in the transgenic mouse. This phenotype is due to a defect in secondary fiber differentiation that sets in shortly after birth. By contrast, lens development is almost unaffected by the expression of a mutant human p53 that is impaired in DNA binding. The phenotype caused by the wild-type human p53 gene can be rescued by the mutant allele, possibly via a mechanism of dominant negative interference. The results have implications for schemes of p53 gene therapy in patients.

Altered striatal functions in a mutant mouse lacking D1A dopamine receptors. Mice homozygous for the deletion carry D1A striatal neurons that are totally devoid of functional D1A receptors. The animals die shortly after weaning unless their diet is supplemented with hydrated food. Although locomotion appears unaffected, the mice display a significant decrease in rearing behavior. Details of the involvement of D1A dopamine receptors in the pathogenesis of Parkinson's disease and schizophrenia, and their role in cocaine and amphetamine addiction can be analyzed with the help of this mouse strain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-01800-06 LMGD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Genetic Basis of Mammalian Kidney Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gregory Dressler, Ph.D.,LMGD, Head, Unit of Molecular Embryology

Others:

Larry T. Patterson	IRTA Fellow	LMGD, NICHD
Gabriella Ryan	IRTA Fellow	LMGD, NICHD
Dawn E. Phelps	Pre-IRTA Fellow	LMGD, NICHD

COOPERATING UNITS *(if any)*

Urologic Oncology Section, Surgery Branch, NCI (J.Gnarra); The Wistar Institute, Philadelphia, PA (F.J. Rauscher)

LAB/BRANCH

Laboratory of Mammalian Genes and Development

SECTION

Section on Molecular Embryology

INSTITUTE AND LOCATION

NICHD, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4 Years	4 Years	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mammalian kidney is used as a model to study the genetic basis of development and oncogenesis in a complex multicellular tissue. Current studies continue to focus on the role of transcription factors in early kidney development and their regulation by potential tumor suppressor genes. Our major goals are to understand the molecular mechanisms through which developmental regulators affect complex morphogenetic pathways. Previously, we have determined that the Pax-2 gene is required for the conversion of early kidney mesenchymal cells to an epithelial phenotype, that the Pax-2 was persistently expressed in human embryonal kidney tumors, and that persistent expression in transgenic mice inhibits terminal differentiation of renal epithelial cells resulting in a condition similar to human nephrotic syndrome. More recently, progress has been made in several new areas.

A. Development. Although Pax-2 is required for early kidney development, whether it is sufficient to initiate the conversion of mesenchymal cells to epithelium has not been determined. Thus, we designed a novel experiment using Pax-2 transducing retroviruses to introduce the active gene into the mesenchymal cells prior to endogenous Pax-2 activation. Preliminary results indicate that expression of Pax-2 is enough to convert the mesenchyme cells into an early epithelial like structure. These results suggest a master regulatory function for Pax-2 in the specification of epithelial cells in the kidney.

B. Oncogenesis. Expression of Pax-2 is repressed during the course of normal renal epithelium development. However, in embryonal kidney carcinomas, such s Wilms' tumor, and in adult renal cell carcinoma Pax-2 expression levels are still high. We have been able to demonstrate that the suppression of Pax-2 during kidney development is mediated, in part, by the Wilms' tumor suppressor gene, WT1. There is a direct correlation between decreasing levels of Pax-2 protein and increasing levels of WT1 in the developing nephron. Furthermore, WT1 binds at least three sites in the Pax-2 5' regulatory sequences and can repress transcription in vitro. Thus, we have established a link between a developmental regulatory gene and a known tumor suppressor gene. In adult tumors, reactivation of Pax-2 may be a necessary determinant for oncogenesis. Inhibition of Pax-2 gene expression by antisense methods results in growth inhibition of renal cell carcinoma derived cell lines. These results suggest a function for Pax-2 in the proliferation of the early epithelial cell, a process concurrent with differentiation.

C. Biochemistry. In order to understand the function of the Pax family of transcription factors, identifying the potential target genes is mandatory. Using Pax-2 specific antibodies, DNA-protein complexes were precipitated from intact chromatin and the DNA binding sequences cloned. DNA footprinting and methylation protection experiments clearly demonstrate Pax-2 binding to these genomic loci. These genomic tags can be used to identify larger genomic clones that may contain potential target genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HD-01801-05-LMGD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Molecular Analysis of Murine Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

K.A. Mahon, Ph.D., Expert
D.E. Feltner, M.D., Guest Worker
E. Hermesz, Visiting Associate
L. Kos, Ph.D, Visiting Fellow
M.A. O'Reilly, Ph.D., NRC Fellow

COOPERATING UNITS *(if any)*

CBER, FDA (M. Jamrich), LP, NCI (S. Mackem), LMG, NICHD (T. Sargent), DEB,NICHD (L. Neiman), U. of Michigan (S.Camper)

LAB/BRANCH

Laboratory of Mammalian Genes and Development

SECTION

Unit on Developmental Gene Regulation

INSTITUTE AND LOCATION

National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Homeobox genes occupy key positions in the regulatory gene hierarchy responsible for establishing the embryonic body plan in *Drosophila*, and are thought to play analogous roles in the development of higher vertebrates. The homeobox gene *Rpx* (for Rathke's pouch homeobox) is expressed during gastrulation in the prospective anterior neural plate, and at later stages in Rathke's pouch, the primordium of the anterior and intermediate lobes of the pituitary. Expression in Rathke's pouch is extinguished between 13.5 and 14.5 days of embryogenesis, coincident with the differentiation of pituitary-specific cell types. Using *lacZ* reporter genes, it has been found that proper spatial and temporal expression in the anterior neural plate can be recapitulated in transgenic mice by as little as 600 bp of upstream sequence and the first intron. Additional elements are required for expression in Rathke's pouch. In addition, an element has been uncovered that directs transgene expression to a region of the hypothalamus and incipient posterior lobe that is in direct contact with Rathke's pouch. *In vitro* tissue recombination experiments have established that this expression is "induced" by contact with the pouch. It is proposed that this element may be present in other genes that normally respond to signals emanating from the pouch during the determination of the hypothalamic-pituitary axis.

The role *Rpx* plays in pituitary development has been investigated in gain-of-function mouse models. Persistent expression in the pituitary has been achieved by directing expression of *Rpx* in transgenic mice with pituitary-specific promoters. Transgenic mice bearing α-glycoprotein subunit promoter-*Rpx* fusion genes express *Rpx* in the pituitary after the endogenous gene normally turns off. These mice are dwarfed and have underdeveloped reproductive tracts. Their pituitaries are very small and are deficient in gonadotroph, thyrotroph, lactotroph, and somatotroph cell lineages. These results indicate that the *Rpx* gene product must be downregulated during development for proper differentiation of the pituitary. The phenotype of these mice is similar to existing dwarf mutants suggesting that Rpx mis-expression may be causatively involved in the genesis of certain types of pituitary dysfunction in mouse and man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

-02

Z01-HD-01803-LMGD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic analysis of Thymocyte Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul E. Love, MD, PhD; Investigator (PHS), LMGD, NICHD
Connie L. Sommers, PhD; IRTA Fellow, LMGD, NICHD
Kun Huang, MS; Biologist, LMGD, NICHD
Daniel A. Charlick, Pre-IRTA Fellow, LMGD, NICHD
Janice Anderson, Pre-IRTA Fellow, LMGD, NICHD

COOPERATING UNITS *(if any)*

CBER, FDA (E.W. Shores) STR,LMGD, NICHD (H. Westphal, A. Grinberg, E.J. Lee)
LEI, NCI (A. Singer, D.L. Weist)

LAB/BRANCH

Laboratory of Mammalian Genes and Development

SECTION

Unit on Cellular and Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH 9000 Rockville Pile, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.75	PROFESSIONAL: 1.75	OTHER: 2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research is directed at understanding the cellular and genetic events that control normal T cell development. Transgenic and gene-targeting methods are used to analyze the function of specific genes in development and various molecular techniques (eg., gene cloning and PCR) are being employed to identify novel genes that participate in thymocyte development. Current studies focus on the role of the T cell antigen receptor(TCR)in thymocyte maturation. The TCR is responsible for antigen recognition, T cell activation and cell mediated immunity. In addition, the TCR plays a critical role in thymocyte development and thymic selection. How the TCR is capable of orchestrating these events remains a central question in developmental immunology. The TCR is composed of multiple signal transducing subunits (the CD3 chains and one or more members of the ζ-family of proteins). Each of these subunits contains sequences that couple the TCR to intracellular signal transduction pathways. To address whether the TCR subunits perform distinct or analogous functions in development several experimental approaches have been taken: a)the function of individual TCR subunits has been examined by overexpression in transgenic mice; b)the role of ζ-chain in T cell ontogeny has been examined by generating ζ-deficient mice, and c)the role of individual ζ-chain signaling motifs or of other ζ-family proteins in thymocyte development is being addressed by transgene-reconstitution of ζ-deficient mice. These studies have led to 2 major findings: 1)Expression of ζ chain (or a related protein) is required for TCR expression and consequently for normal T cell development. However, surprisingly, signals transduced by ζ are not absolutely required for T cell development. Thus the CD3 module of the TCR is sufficient to transduce all of the signals necessary for thymocyte maturation. 2)Individual signal transducing molecules (i.e., the CD3 chains and the ζ-family proteins) function at different stages of development, presumably as subunits of distinct signaling complexes. The composition of these complexes and their function in development is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01310-08 LMGR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Gene Regulation of the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI: K. Ozato Head, SMGI LMGR, NICHD

See Attached

COOPERATING UNITS (if any)

Unit on Molecular Mechanisms of Reproductiion (Dr. James Segars)

LAB/BRANCH

Laboratory of Molecular Growth Regulation

SECTION

Section on Molecular Genetics of Immunity

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 12.6	PROFESSIONAL: 9.4	OTHER: 3.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This group works on gene regulation in the immune system. Our focus has been to elucidate the role of two transcription factors, RXRß and ICSBP that we have isolated previously. RXRß is a member of the nuclear hormone receptor superfamily and by heterodimerizing with retinoic acid receptor (RAR), it regulates retinoic acid (RA) responsive genes. ICSBP is a member of the interferon regulatory factor (IRF) family and is involved in viral/interferon (IFN) mediated gene regulation. To further study the role of ICSBP and other members of the IRF family in the immune system, a dominant negative ICSBP has been constructed which has only the DNA binding domain (DBD) and lacks a large C-terminal domain. This construct was stably transfected into a human monocytic cell line U937 cells, and a number of clones expressing the DBD have been established. The DBD clones exhibited altered infectivity by several viruses: they were refractory to infection by vaccinia virus, ectromeria virus as well as human immunodeficiency virus-1. In contrast, control clones and those expressing the intact ICSBP were strongly infected by these viruses. The DBD clones were, however, infected by vesicular stomatitis virus (VSV) as efficiently as control clones. But, IFN pretreatment known to confer protection against VSV, completely failed to protect DBD clones. As expected control clones and those expressing intact ICSBP were fully protected from VSV infection by IFNs. These results indicate that some viruses depends on the IRF proteins for their infection and growth in host cells.
By genomic footprinting analysis we have studied binding of RXR heterodimers to the promoter of an RA responsive gene (RARß) in P19 EC cells. We show that the RA responsive element (RARE) in the RARß gene is not occupied prior to RA treatment, but becomes occupied within 1 h of RA treatment, without requiring new protein synthesis. This in vivo occupancy accompanied factor binding to other elements in the promoter. The occupancy at all sites was rapidly reversed when RA was withdrawn from culture medium. On the other hand, P19 cells expressing a dominant negative RXRß that blocks binding of endogenous RAR/RXR heterodimers to the RARE, did not exhibit occupancy at all elements in the promoter. Our observations underscore the significance of ligand for receptor/DNA intereaction in vivo and demonstrate the presence of a hierarchy in factor binding processes.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 08719-14 LMGR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development and Uses of Eukaryotic Vectors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

PI:	Bruce H. Howard	Chief	LMGR,NICHD
Other:	M.L. Avantaggiati	Visiting Fellow	LMGR,NICHD
	C. Driscoll	Biologist	LMGR,NICHD
	V. Ogryzko	Visiting Fellow	LMGR,NICHD
	E. Englander	Visiting Associate	LMGR,NICHD
	G. Humphrey	Sr. Staff Fellow	LMGR,NICHD
	V. Russanova	Visiting Fellow	LMGR,NICHD
	K. Engleka	NRC Bio Tech	LMGR,NICHD

COOPERATING UNITS (if any)

Frank A. Suprynowicz, LTPB,NICHD

LAB/BRANCH

Laboratory of Molecular Growth Regulation

SECTION

Section on Human Cell Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	6	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on Alu repetitive sequences have further established that these elements are maintained in a transcriptionally silent state by complex and potentially redundant mechanisms. Concurrent investigation of the retinoblastoma tumor suppressor gene product (pRb) led to discovery that this protein can modulate exit from mitosis as well as G1 to S phase transit. Together, Alu and Rb studies prompted formulation of a model in which heterochromatin maintenance serves as an unconventional and previously unappreciated cell cycle control mechanism that may be compromised in senescent cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00412-07 LMGR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Regulation of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.:	Richard J. Maraia	Senior Research Investigator	LMGR, NICHD
Other:	D.-Y. Chang	NRC BioTech Fellow	LMGR, NICHD
	J. Sarrowa	IRTA	LMGR, NICHD
	N. Sasaki-Tozawa	EBON	LMGR, NICHD
	K. Hsu	HHMI-Research Scholar	

COOPERATING UNITS (if any)

Department of Pathology, Texas Children's Hospital (Gretchen Darlington)
Department of Microbiology/Immunology, Duke Univ. Medical Center (Jack Keene)
Washington University, St. Louis (Eric D.Green)

LAB/BRANCH

Laboratory of Molecular Growth Regulation

SECTION

Section on Human Cell Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research on repetitive genetic elements of mammals was continued with emphasis on human Alu elements and other small RNA-encoding sequences. Alu sequences are small transposed elements endogenous to the human genome. Nearly one million dispersed copies constitute about 5% of our genetic material. Alu mobility causes genetic variability and heritable disorders. The regulated expression of Alu and its mouse homologue B1 into small RNA was studied. Germline and fetal-specific expression suggests their involvement in development. Examination of mechanisms of B1 and Alu RNA expression continued to yield insight into their complex regulation and propensity for transposition. Advances in understanding regulation of efficiency of transcription by RNA polymerase III were made. 1) A transcription termination and reinitiation factor for RNA polymerase III, previously identified as the human autoantigen La, was further characterized. 2) Human cDNAs for RNA bidning proteins that modulate Alu RNA metabolism were isolated and characterized. 3) The gene encoding a trinucleotide repeat-containing Alu RNA binding protein was mapped to human chromosome 15q22. 4) The gene for all small cytoplasmic Y4 RNA gene was cloned, mapped to human chromosome 6 and its promoter elements functionally characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00505-01 LMGR

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Eukaryotic Transcriptional Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute

P.I.:	Yoshihiro Nakatani	Visiting Scientist	LMGR, NICHD
Other:	Tetsuro Kokubo	Visiting Fellow	LMGR, NICHD
	Kaname Saida	Special Volunteer	LMGR, NICHD
	Junichi Nishikawa	Special Volunteer	LMGR, NICHD

COOPERATING UNITS (if any)

Laboratory of Biochemistry and Molecular Biology, The Rockefeller University (Robert G. Roeder)

LAB/BRANCH

Laboratory of Molecular Growth Regulation

SECTION

Section on Human Cell Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transcription of protein-encoding genes in eukaryotes is regulated by various gene-specific transcriptional activators by interacting with RNA polymerase II transcription machinery. Transcription initiation factor TFIID is a potential target for activators and plays a central role in this communication. TFIID is a multisubunit complex containing a TATA box-binding factor (TBP) and associated polypeptides (TAFs). Importantly, while the native TFIID complex mediates both basal and activator-dependent transcription in reconstituted systems, TBP itself is competent only for basal transcription. Thus, TAFs are essential cofactors for regulated transcription. Recently, we have cloned most of TAFs with oligodeoxynucleotide probes based on partial amino acid sequences. The largest subunit, p230, shows significant sequence similarity with the product of human cell cycle gene (CCG1), also identified as the largest subunit of human TFIID complex, suggesting a specific role for a part of p230 in cell cycle regulated transcription. Moreover,p230 N-terminal portion interacts directly with TBP and inhibits both TATA box binding and basal transcription activities in the absence of other TAFs, suggesting that p230 negatively regulates promoter binding of TFIID complex. The second subunit, p110 interacts directly with p230 N-terminal portion which inhibits TBP function, suggesting a functional communication between p110 and TBP via p230 interactions. The third subunit, p85 contains eight of the WD-40 repeats found originally in the beta-subunit of G-proteins and more recently in other transcriptional regulatory factors. The WD-40 repeats in p85 may play a significant role in transducing signals by interactions with transcriptional regulators and/or other components of the basic transcriptional machinery. The p62 and p42 subunits have sequence similarities with H4 and H3 core histones, respectively, and interact tightly each other via histone-like domains. One interesting possibility is that p62/p42 interactions with DNA could facilitate the displacement of nucleosomal histones H3/H4 and/or maintain a compacted DNA structure near the transcription start site. Two small TAFs (p28, p22) are synthesized from the single gene by alternative splicing. Closely related TAFs are suggestive of TFIID heterogeneity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00165-19 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and Characterization of Macromolecular and Cellular Particles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Chrambach	Head	SMA, LTPB, NICHD
OTHERS:	M. M. Garner	Expert	SMA, LTPB, NICHD
	S. P. Radko	Courtesy Associate	SMA, LTPB, NICHD
	A. Sokoloff	Visiting Associate	SMA, LTPB, NICHD
	G. Caiafa	Collaborator	SMA, LTPB, NICHD
	P. G. Johnston	Collaborator	NCI-NMOB, DCT, NCI

COOPERATING UNITS *(if any)*

NCI-NMOB, DCT, NCI (Patrick G. Johnston)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) Aiming at detecting of conformational and/or net charge changes at the microsome surface upon initiation of fusion, predicted on the basis of literature reports of protein alterations, rat liver microsomes were fractionated by capillary electrophoresis in polymer solutions serving as media for "particle sieving" (separation based on size and shape differences). In non-borate buffer containing optimally resolving polymers, the purified microsome preparation appeared homogeneous. The size of the microsome was estimated in those media as 16 +/- 6 nm geometric mean radius. Upon addition of Mg++ and either GTP-gamma-S (no fusion) or GTP (fusion), several new microsome components are separated. However, their sizes and net charges are not measurable by capillary electrophoresis since they remain unretarded in the water soluble range of available polymers, consistent with the behavior of very large particles. 2) Cisplatin treatment of cells results in a shift from large molecular weight DNA populations to smaller ones. In an attempt to monitor that shift for the purpose of predicting the success of clinical cisplatin treatment, an electrophoretic method for distinguishing DNA populations differing in size was developed, using thymus DNA sonicated for various times as models for such populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00171-18 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electrophoretic Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Chrambach	Head	SMA, LTPB, NICHD
OTHERS:	M.M. Garner	Expert	SMA, LTPB, NICHD
	S.P. Radko	Courtesy Associate	SMA, LTPB, NICHD
	S.F. Zakharov	Visiting Fellow	SMA, LTPB, NICHD
	E. Yarmola	Adjunct Scientist	SMA, LTPB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) The upper molecular size limit amenable to separations by capillary electrophoresis in polymeric molecular sieving media was sought in order to delineate the feasibility of cell fusion investigations by this method. Using polystyrene size standards, that upper limit was found to be 10 um in geometric mean radius which appears sufficient for our purpose. 2) Recently developed commercial automated gel electrophoresis apparatus with intermittent scanning of fluorescence was evaluated with respect to 4 questions: a) Is it applicable to native DNA in spite of fluorescent labeling by intercalation of a dye? b) Is it capable of providing band width and shape data that provide information about molecular conformation, association state and microheterogeneity? c) Is it possible to define a nonlinear Ferguson plot (serving to interpret mobilities to provide information on molecular and gel fiber properties) with one or two electrophoretic runs in the 8 gel lanes of the apparatus containing 8 gel concentrations? d) Can it serve as a general preparative device at the microgram scale? We were able to provide the evidence for an affirmative answer to all of the four questions. 3) The previously demonstrated capacity of uncrosslinked polyacrylamide gels to resolve a 350 bp DNA fragment with a single base mismatch from the normal control prompted the question whether un- or very low-crosslinked polyacrylamide exhibited an enhanced resolving capacity toward DNA in general. This is not the case. 4) The function relating electrophoretic retardation of albumin with the molecular weight of polymer was found to be triphasic. The rise of retardation in solutions of polymers in excess of 1 million molecular weight coincides with the sharp increase in viscosity indicative of the formation of a polymer network.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01501-03 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Lipids in Membrane Rearrangements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	L. Chernomordik	Visiting Scientist	LTPB, NICHD
OTHERS:	A.N. Chanturiya	Visiting Associate	LTPB, NICHD
	E.A. Leikina	Visiting Associate	LTPB, NICHD
	Myoung-Soon Cho	Biologist	LTPB, NICHD
	J. Green	Summer IRTA	LTPB, NICHD
	J. Zimmerberg	Chief	LTPB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Membrane and Cellular Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3 1/2	2 1/4	1/4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To fuse, membranes must bend. The energy of the unknown bent intermediates of biological fusion in diverse physiological processes depends on the ability of membrane lipids support the required curvatures. We have studied the effects of membrane lipid composition on the rates of fusion reaction. Lipids of the different molecular shapes such as inverted cone-shaped lysophosphatidylcholine, LPC, and cone-shaped arachidonic acid, AA, were added exogenously to membranes to alter their propensity to bend. Addition of LPC to contacting monolayers of cell membranes inhibited low pH triggered cell-cell fusion mediated by the specialized envelope proteins of baculovirus (gp64) and influenza virus (hemagglutinin). In contrast, AA promoted these fusion reactions. Modulation of membrane lipid composition reversibly affected fusion (inhibited or promoted) at a stage of actual merger of membrane lipid bilayers which follow the triggering of fusion. To elucidate the mechanisms of lipid bilayer fusion in the well defined model system we studied the fusion of phospholipid vesicles to planar phospholipid bilayer membranes. Two distinct fusion stages, hemi- and complete fusion (i.e., membranes' lipid mixing without and with fusion pore formation, respectively) were established. Hemifusion was prevented by adding inverted cone-shaped LPC to contacting monolayers of two fusing membranes. In contrast, fusion pore formation depended on the composition of distal monolayers: LPC promoted and cone-shaped AA inhibited complete fusion. Thus, lipids differentially modulate membrane fusion by stages according to lipid shape and place. The similarity between effects of the membrane lipid composition obtained for fusion of model lipid membranes and those for biological membrane fusion supports the hypothesis that stalk (local connection between two membranes leading to membrane hemifusion) and pore types of fusion intermediates may be involved in the biological fusion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01400-12 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dynamics of the Growth and Development of Bone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.L. Yergey, Ph.D.	Head	SMAMS, LTPB, NICHD
OTHERS:	N. Vieira	Biologist	SMAMS, LTPB, NICHD
	R. Goans, M.D., Ph.D.	Senior Staff Fellow	SMAMS, LTPB, NICHD

COOPERATING UNITS *(if any)*

PSL, DCRT (G. Weiss); Human Genetics Branch, NICHD (J. Marini); Child Nutrition Research Center, Houston, TX (S. Adams); Nemours Children's Clinic, Jacksonville, FL (N. Mauras)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Development of new kinetic models. a. Using results from isotope dilution experiments with intravenous ^{42}Ca in humans, a three hour test has been developed to measure the short-term clearance of calcium. This clearance reflects bone uptake and the state of local calcium dynamics at the bone surface. In studies of 91 normal female subjects, ages 4-50 years as well as 41 subjects with various forms of metabolic bone disease, results of this approach have been found to correlate with bone accretion measured from classical calcium kinetic studies with a correlation coefficient of 0.721, significant at $p<0.005$. b. A second novel model to describe calcium isotope dilution observations is a generalized non-Markovian model describing the long-term kinetics of calcium interaction with bone. In this model the reversible component of calcium flow to bone is modeled by a retention function. Using children with juvenile dermatomyositis, reversible loss to bone is found to be the same between the steroid and non-steroid treated subjects while irreversible tracer loss from plasma to bone is found to be statistically reduced ($p=0.049$) in glucocorticoid-treated patients relative to those untreated. 2. Ultrasonic imaging of the calcaneus has been performed as a potential alternative imaging approach to bone histomorphomorphometry of cancellous bone. Measurements in 142 healthy male and female subjects in the age range 18 months-84 years show a clear falloff in scattered ultrasound intensity in both males and females after the age of 35. The rate of fall in females is more pronounced than that of males. 3. The dynamic equilibrium calcium atom exchange between solution and crystal was studied using a slurry of $CaCl_2$ and NaH_2PO_4 mixed with 50μm diam hydroxyapatite crystals in which initially 5% of the Ca ions in solution were ^{42}Ca. The time course of the isotope both in solution and on the crystals was measured for 24 hrs using thermal ionization and ion microprobe mass spectrometry respectively. Isotope disappeared from the filterable portion of the slurry in the time of the study, diminishing by about 20% during the day, and at the same time, isotope was seen to accrete on the surface of the crystalline material isolated by filtering, with the isolated crystals being found uniformly labelled on their surfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01401-12 LTPB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Applications of Liquid Chromatography/Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Yergey	Head	SMAMS, LTPB, NICHD
Others:	K. O'Brien	IRTA Fellow	SMAMS, LTPB, NICHD
	X. Zhang	IRTA Fellow	SMAMS, LTPB, NICHD

COOPERATING UNITS *(if any)*
Developmental Endocrinology Branch, NICHD (F. Cassorla, K. Weiner, K. Oerter); Dept. of Nutrition, Univ. of Connecticut, Storrs, CT (L. Allen, M-L. Caldera)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.3	PROFESSIONAL: 2.3	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01408-04 LTPB

PERIOD COVERED
October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stability and Specificity of DNA-Protein Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Garner	Expert	LTPB, NICHD
Others	A. Chrambach	Chief	SMA, LTPB, NICHD
	J. Zimmerberg	Chief	LTPB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Biochemistry and Metabolism, NIDDK (D.C. Rau)
Laboratory of Molecular Biology, NCI (S. Adhya)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Macromolecular Analysis

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

DNA binding proteins bind to DNA in both sequence specific and nonspecific modes in vivo and in vitro. The extent of gene expression is controlled by the extent to which the protein overcomes the competing nonspecific DNA and binds to its specific site. Various physiologically relevant factors may affect the ratio of specific to nonspecific binding. This project is attempting to determine the role of hydration and water release in the stability and specificity of recognition. When the two macromolecules bind, some of the interactions with the milieu are lost, and new interactions with the complementary surface are formed. This exchange of interactions with water and ions for interactions with the other macromolecule makes the extent of binding very sensitive to solution conditions. In addition, DNA-mediated protein-protein interactions (e.g. DNA looping), are an important part of the biological activity of most transcriptional regulators. By quantitating the binding in the presence of high concentrations of small molecule solutes, one can determine the effect of surface hydration of both protein and DNA on complex formation, and therefore the total number of water molecules released in the binding reaction, which should be related to the amount of surface area of both protein and DNA occluded in the complex. Such insight into the hydration of both surfaces before and after complexation is important for understanding the sources of stability and specificity of a protein-DNA complex. We have shown that this water release phenomenon occurs only for specific binding, which suggests a mechanism for gene regulation in response to high osmotic stress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01409-09 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membrane Transport and Fusion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Zimmerberg	Chief	LTPB, NICHD
Others:	P. Blank	IRTA Fellow	LTPB, NICHD
	E. Suss-Toby	Visiting Fellow	LTPB, NICHD
	I. Plonsky	Visiting Fellow	LTPB, NICHD
	V. Ratinov	Adjunct Scientist	LTPB, NICHD
	E. Ratinov	IRTA Fellow	LTPB, NICHD
	M-S Cho	Biologist	LTPB, NICHD
	G. Ward	Senior Staff Fellow	LMR, NIAID

COOPERATING UNITS *(if any)*

Department of Physiology, Rush Medical College, Chicago, IL
Laboratory of Malaria Research, NIAID

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	5.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our research on fusion, the fundamental step in viral and parasitic infection, fertilization, secretion, and neuro-transmission. By developing an extensive set of computer programs, we can now measure the fusion pore conductance in a number of different ways that take more experimental parameters into account than possible previously. In addition, we developed methods for measuring very fast latencies between the time of solution application and fusion pore opening. With direct current, double whole-cell recording, measurement of the fusion pore is model-independent. Using this technique in baculovirus-induced cell-cell fusion, a large distribution of individual fusion pore conductances was measured with cell capacitance, and found to be statistically indistinguishable from that measured by direct recording, thus further validating the capacitance technique. We found that the latency to fusion after triggering was distributed about a mean of 627 ms, much shorter than that of influenza virus. This suggests either a higher fusion protein density or the absence of an appreciable delay, usually attributed to fusion protein aggregation. Preliminary experiments on the electrophysiology of invasion of cells with the protozoan parasite *Toxoplasma gondii* indicate that the initial response of the host cell is a transient increase in cell membrane conductance. A new preparation for measuring sperm-egg fusion pores during fertilization is currently being developed.

Using light-scattering, we are studying the sea urchin planar isolated cortex because it is the only model system that exists for studying exocytosis in which fusion kinetics are not initially substrate limited, since cortical granules are both predocked to the target membrane and in great abundance. In both *Lytechinus pictus* and *Strongylocentrotus purpuratus*, we found stable, sub-maximal responses with micromolar concentrations of calcium, with no further fusion at that calcium concentration regardless of mode, duration, or frequency of calcium application. Rather, more fusion was always seen upon raising calcium concentration. Thus the calcium sensitivity of secretory granule exocytosis in vitro is heterogeneous.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 14015-04 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Exocytotic Membrane Fusion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Vogel	Senior Staff Fellow	LTPB, NICHD
Others:	J. Zimmerberg	Chief	LTPB, NICHD
	P. Blank	IRTA Fellow	LTPB, NICHD
	T. Whalley	Visiting Fellow	LTPB, NICHD
	D. Lester	Staff Scientist	FDA
	M. Terasaki	Senior Staff Fellow	LN, NINDS
	S. Beushausen	Visiting Scientist	LN, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Following the fertilization of sea urchin eggs, calcium is released from intracellular stores triggering the fusion of thousands of exocytotic cortical granules with the egg plasma membrane. We have used this preparation to study several aspects of calcium triggered exocytosis. 1) To identify proteins involved in exocytosis, we have used a reversible inhibitor of triggered exocytosis, biotin-HPDP. With this reagent, we have biotinylated granule proteins and purified protein substrates using immobilized avidin chromatography. We have isolated the major cortical granule substrate of biotin-HPDP and are currently sequencing it. Preliminary sequence data suggests that it is an as yet unidentified protein. 2) We have developed a stochastic computer simulation to model the calcium dependent behavior of triggered exocytosis in our system. We have now developed a closed form equation which describes the behavior of our simulation, and for the first time we have a model based on first principles to explain the extent and rate of calcium triggered exocytosis. 3) We have also studied the relationship between exocytosis and endocytosis in sea urchin eggs. Cortical granule exocytosis in sea urchin eggs is so massive that it transiently doubles the egg's membrane surface area while simultaneously eliminating ~7% of the egg's volume. We find that the eggs can compensate for these dramatic changes in surface area and volume by directly internalizing large 1.5 μm diameter membrane delineated structures subsequent to exocytosis. Our data suggests that these structures are formed by the re-sealing of exocytotic fusion pores. Finally, 4) We have developed a 96-well microtiter dish assay using sea urchin egg plasma membrane-cortical granule fragments, to screen drugs for their ability to lyse membranes, perturb membrane curvature, and interfere with triggered exocytosis. Preliminary findings from screening 17 different drugs with this assay have been the identification of a widely used anti-cancer drug which reversibly inhibits exocytosis at a step prior to calcium triggering; and another anti-cancer drug which perturbs granule membranes, resulting in lysis and fusion in the absence of calcium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01416-02 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tissue Imaging in Cell Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Zimmerberg	Chief	LTPB, NICHD
Others:	L. Margolis	Courtesy Associate	LTPB, NICHD
	S. Glushakova	Courtesy Associate	LTPB, NICHD
	B. Baibakov	Courtesy Associate	LTPB, NICHD

COOPERATING UNITS *(if any)*

Duke University (S. Simon); NCI (Stettler-Stevenson)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continuing to develop the technique of imaging cells in three-dimensional long-term histocultures of mammalian tissues, we have applied both standard and newly developed methods to the study of cell-cell interaction inside stratified rat lingual epithelium and to the invasion of normal tissue by melanoma cells in vitro. By using confocal microscopy in combination with histocultures of stratified rat lingual epithelium we have proved the existence of gap junctions between cells of one stratum as well as between strata. Neural cells PC12 differentiate in blocks of stratified epithelia without additional growth factors. The neurites formed by PC-12 cells establish contacts with epithelia.

Imaging of genetic variants of mouse melanoma cells implanted into histocultured mouse lung showed that the pattern of invasion correlates with the activity of metalloproteinase on the surface of the invading cell. The overproduction of the protease inhibitor on the surface of melanoma cells results in dissemination of single cells through the normal tissue. This pattern of invasion correlates with the decrease of cell-cell adhesion in cell suspension. These systems provide the opportunity to study the mechanisms of normal and pathological cell-cell interactions in a native-state microenvironment as well to test various drugs affecting these interactions.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01417-01 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Energetics of the Interaction Between Water, Membranes, and Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A.L. Yergey	Head	SMAMS, LTPB, NICHD
Others:	Y. Xu	IRTA Fellow	SMAMS, LTPB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Measurement of the enthalpy change for the successive additions of water molecules to a hydrated proton cluster have been studied using an adaptation of an existing thermospray ionization source. Results to date show that clusters with as many as 7 water molecules can be observed, and that the relative intensities of the clusters observed are consistent with ion source pressures determined by direct measurement. At present, we observe rather substantial changes in K_{eq} for sequential additions as a function of pressure. Because the ion intensities of the clusters observed are reasonably consistent with direct measurement of source pressures, we believe that the pressure dependent changes in K_{eq} are a consequence of perturbations external to the ion source. In particular, we feel this is a consequence of ion optic electric fields inducing collisional dissociation of clusters. 2. We have taken the Cu(II) glycine and glycyl-glycine interactions as a model of metal/peptide interaction. Complexes of $[\mathrm{Cu(II)}\text{<-}\mathrm{gly}]^+$ and $[\mathrm{Cu(II)}\text{<-}\mathrm{gly\text{-}gly}]^+$ and $[\mathrm{gly}\text{->}\mathrm{Cu(II)}\text{<-}\mathrm{gly\text{-}gly}]^+$, having the correct $^{63}\mathrm{Cu}/^{65}\mathrm{Cu}$ isotope ratio, are observed in the electrospray spectrum of solutions of these species. These singly charged ions can be explained on the basis of the presence of gly and gly-gly as anions in solution, rather than as complexes of neutral gly or gly-gly formed in the gas phase. The solution ions are postulated to be 5-membered ring structures resulting from complexes of the Cu(II) ions and non-bonding electrons of the amino acid residues. If such ions were formed in a gas phase reaction after electrospraying, one would expect to see either doubly charged ions corresponding to a Cu(II)-gly complex, i.e., no anion, and/or singly charged ions having an additional proton, corresponding to a Cu(II)-gly. Therefore we have both a model of solution processes and proof of the origin of at least some ions of electrospray mass spectrometry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01418-01 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Mitosis at the Cellular Level

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	F. Suprynowicz, Ph.D.	Senior Staff Fellow	LTPB, NICHD
Others:	T. Whalley, Ph.D.	Visiting Fellow	LTPB, NICHD

COOPERATING UNITS *(if any)*

Laboratory of Molecular Growth Regulation, NICHD

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The onset and completion of cell division (mitosis) are controlled by activation and inactivation of the cdc2 protein kinase. Molecular analyses have shown that cdc2 kinase activity is regulated in a complex manner involving multiple phosphorylations and the physical association of cdc2 with mitotic cyclins, proteins which oscillate in abundance over the course of the cell cycle. It is generally thought that inactivation of the cdc2 kinase directly results from the destruction of mitotic cyclins. To understand the pathways controlling cdc2 kinase activation and inactivation at the cellular level, we have developed an approach that combines techniques of biochemistry and cell physiology using permeabilized cells and cell-free assays. We have shown that physiological levels of Ca2+ can inactivate the cdc2 kinase in the absence of cyclin destruction, suggesting the hypothesis that a Ca2+-dependent mechanism initiates inactivation, and that the destruction of cyclin serves to ensure that another round of kinase activation does not take place before another round of DNA replication. In many cells, the activation of protein kinase C (PKC) leads to a reduction of intracellular Ca2+ levels. We have shown that the activation of PKC blocks cdc2 kinase inactivation without blocking the destruction of mitotic cyclins. This finding further demonstrates that cdc2 kinase inactivation and cyclin destruction can be uncoupled, and supports the view that intracellular Ca2+ levels are a key component of the pathway controlling exit from mitosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01419-01 LTPB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Localization of Membrane Fusion Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Sokoloff	Visiting Associate	LTPB, NICHD
OTHERS:	T. Whalley	Visiting Fellow	LTPB, NICHD
	S. Radko	Courtesy Associate	LTPB, NICHD
	J. Zimmerberg	Chief	LTPB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Membrane and Cellular Biophysics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

GTP-triggered fusion of rat liver microsomes derived from the endoplasmic reticulum was studied as a model for intracellular membrane fusion. We showed that the fusion is insensitive to pretreatment of microsomes with ATP and that the fusion of N-ethylmaleimide (NEM)-inactivated microsomes can not be restored by the addition of untreated cytosol. Treatment of microsomes with high salt did not inhibit fusion. These results are in contrast to those obtained in studies of reconstructed secretory and endocytic steps mediated by membrane fusion and suggest that the fusion of microsomes involves different proteins or combinations of proteins. Similar results have been obtained studying membrane fusion in the sea urchin egg cortical reaction. The NEM-sensitive fusion-related thiol groups in the membrane-bound microsomal fusion machinery have been characterized using bulky reagents prepared by the introduction of 3-(2-pyridyldithio)propionate (PDP) groups into BSA or aminodextrans. The reagents inhibited microsome fusion by reacting with thiol groups. The reaction was reversed by dithiothreitol. It has been found that modification of microsomes with these reagents protected fusion activity against inhibition by NEM. This indicates that fusion-related thiol groups are located on the outer side of the microsomal membrane and are accessible to macromolecules from the medium and, perhaps, to components of the opposite membrane in membrane fusion. The possibility that the exposed thiols belong to the tyrosine phosphatase on the microsome surface, known to have a highly exposed thiol group critical for its activity and suggested to have a regulatory role in assembly and disassembly of the endoplasmic reticulum, was examined. Fusion was completely inhibited by micromolar concentrations of zinc inactivating the phosphatase. However, it was insensitive to pre-treatment of microsomes with a recombinant soluble tyrosine phosphatase and to the presence of an excess of tyrosine phosphate in the medium. The application of the reagents to the sea urchin egg exocytotic apparatus gave very similar results. Based on these findings, we synthesized a reagent suitable for isolation of modified proteins. For this purpose, dinitrophenol (DNP) groups recognized by specific antibodies were introduced into BSA along with PDP groups. Immunoprecipitation of microsome proteins modified by BSA-PDP-DNP by anti-DNP immobilized monoclonal antibodies revealed a few proteins. Currently, raising monoclonal antibodies against immunopurified microsomal and sea urchin proteins reactive with BSA-PDP-DNP is in progress.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00093-20 OSD

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Action of Nerve Growth Factor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Guroff, Ph.D. Head, SGF OSD, NICHD

Others: (See Attached)

COOPERATING UNITS *(if any)*

(See Attached)

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on Growth Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
11.50	10.00	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nerve growth factor (NGF) is one of the longest known and best characterized of the peptide growth factors acting on the nervous system. It is now recognized as a member of a family of growth factors, called the neurotrophins, now five in number, that supports a wide variety of neural cells. NGF is required for the survival of sympathetic and sensory neurons. It also is involved in the development of several different cell types, including certain neurons in the central nervous system, the chromaffin cells of the adrenal medulla, and a number of tumors, as well. The first step in the action of NGF on these different cells is its binding to a specific, high-affinity receptor, now known to be the protein product of the trk protooncogene. This binding activates the tyrosine kinase activity of trk and initiates a number of intracellular actions that lead to alterations in the phosphorylation and, consequently, the function of key proteins in the cell and to changes in the expression of specific genes. These changes in protein function and in gene expression, caused by the changes in phosphorylation, are the mechanism by which NGF exerts its developmental effects on the cells. Much of the work leading to this concept has been done with the PC12 pheochromocytoma, a cell line derived from a tumor of the rat adrenal medulla. This clonal line continues to be one of the most informative tools available for the study of NGF and a key model for neuronal differentiation in general. In the presence of NGF, PC12 cells stop dividing, elaborate neurites, become excitable, and will synapse with appropriate muscle cells in culture. Indeed, they change from a rapidly-dividing chromaffin cell to a terminally-differentiated sympathetic neuron within a few days. The changes in phosphorylation that underlie these global alterations in phenotype occur in virtually every compartment in the cell. Phosphorylation of NGF-stimulated calcium channels appears to regulate the calcium flux across the membrane and, in turn, the intracellular calcium levels, and these levels surely influence the survival of target neurons and may also regulate the ability of the neuron to withstand environmental insults such as occur in stroke. The NGF-induced phosphorylation of specific transcription factors determines which genes are induced and which are repressed. The role of NGF-induced phosphorylation in the translation of certain NGF-sensitive proteins, such as the epidermal growth factor receptor, is just being explored. Phosphorylation of structural proteins may control the morphological differentiation of neurons. A detailed understanding of the mechanism by which NGF acts will surely illuminate the control of neuronal differentiation and survival. This, in turn, could provide insights into disease states in which neurons either develop inappropriately or die prematurely.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2400-03 PRB

PERIOD COVERED

October 1, 1992 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The role of subclinical infection and cytokines in preterm parturition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberto Romero, M.D.	Head	PB, NICHD
Others:		
Hernan Munoz, M.D.	Visiting Fellow	PB, NICHD
Ricardo Gomez, M.D.	Visiting Fellow	PB, NICHD

COOPERATING UNITS *(if any)*

Department of Obstetrics and Gynecology, Wayne State University (David Cotton, MD, Stanley Berry, MD, and Ruben Quintero, MD)

LAB/BRANCH

Perinatology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, Washington, DC 20007

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to define the role of ascending intrauterine infection in the etiology of preterm labor and delivery, to elucidate the cellular and molecular mechanisms signalling preterm parturition in the setting of infection and to apply this knowledge to improve the diagnostic and therapeutic approach to preterm labor.

The laboratory concluded the following studies:

1. Description of the systemic and intra-amniotic cytokine profile in an animal model of endotoxin-induced preterm parturition: We found that preterm parturition is preceded by an increase in the maternal and amniotic fluid concentration of macrophage derived cytokines. These observations strengthen the hypothesis that cytokines play a role in the mechanisms of infection-induced preterm labor and delivery.

2. Effect of anti-cytokine agents in endotoxin induced preterm labor and delivery: We demonstrated that neither the natural interleukin-1 receptor antagonists nor an anti-tumor necrosis factor antibody are effective in preventing endotoxin-induced preterm labor and delivery in mice.

3. Effect of labor, gestational age, fetal gender and intrauterine infection on the concentrations of the natural interleukin-1 receptor antagonist in the maternal, fetal and amniotic fluid compartments: We demonstrated that the IL-1ra is a physiology component of amniotic fluid as well as maternal and fetal plasma. Detectable IL-1ra was found in the human fetus as early as the 18th week of gestation and infection was associated with an increase in fetal plasma and amniotic fluid IL-1ra concentrations. Female fetuses had a higher urine concentration of IL-1ra than male fetuses.

4. Demonstration that amniotic fluid concentrations of prostaglandins increase early during the process of spontaneous human parturition at term.

5. Demonstration that amniotic fluid prostanoid concentrations are higher in samples obtained from the forebag compartment than from the "upper amniotic compartment".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 2401-02 PRB

PERIOD COVERED

October 1, 1992 to September 30, 1994

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prenatal Diagnosis of Congenital Anomalies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Roberto Romero, M.D.	Head	PB, NICHD
Others:		
Hernan Munoz, M.D.	Visiting Fellow	PB, NICHD
Ricardo Gomez, MD	Visiting Fellow	PB, NICHD

COOPERATING UNITS *(if any)*

Department of Obstetrics and Gynecology, Wayne State University (David Cotton, MD, Stanley Berry, MD, and Ruben Quintero, MD)

LAB/BRANCH

Perinatology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, Washington, DC 20007

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The focus of this project is to improve the diagnosis and treatment of fetal disease. Focus is placed on the prenatal diagnosis of congenital anomalies with non-invasive methods (e.g. high resolution ultrasound and color Doppler flow mapping).

The most significant contribution of the Branch was the performance of the first successful endoscopic surgical procedure in the human fetus. Transabdominal thin-gauge fetoscopy was used to cystoscopically fulgurate posterior urethral valves in a case of urethral obstruction. This procedure represents a new approach to the treatment of selected fetal congenital anomalies, as well as placental conditions amenable to surgical treatment.

The Branch used high resolution ultrasound to establish prenatal diagnosis of conditions heretofore undiagnosed sonographically (e.g. nonrhizomelic chondrodysplasia punctata). Color Doppler flow mapping improved the accuracy of prenatal diagnosis of several other conditions suspected by sonography.

The goal of the Branch is to expand the diagnostic and therapeutic indications of embryofetoscopy, as well as the diagnostic capability of high resolution ultrasound and color Doppler flow mapping.

THE NIH LIBRARY
NATIONAL INSTITUTES OF HEALTH

LIBRARY
NIH
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

AMERICAN BINDERY EAST
2-95

NIH LIBRARY
3 1496 00630 0837